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SOIL MICROBIAL COMMUNITY DYNAMICS
IN FLORIDA SCRUB ECOSYSTEM

by

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Agronomical Engineering. Universidad de Buenos Aires, 2001.

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Biology
in the College of Arts and Sciences
at the University of Central Florida
Orlando, Florida

Spring Term
2005

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I dedicate the present research to my loving family who has always been of great support throughout my career.

Le dedico la presente investigación a mi familia que me ha brindado apoyo y cariño durante toda mi carrera.

ABSTRACT

Pyrogenic ecosystems are maintained by fires which vary in frequency, seasonality, and intensity. Florida oak-saw palmetto scrub ecosystem is characterized by fires occurring at intervals of 10-20 years. Diverse factors as private land acquisition and development has created a patchy distribution of scrub ecosystems and also interrupted the natural fire cycle. The effects of fire over plant regeneration and fauna habitat utilization of the scrub have been well characterized in previous research. In the present paper the objective is to characterize the short- and long-term fire effects on the soil microbial community. Fire effects were studied in a chronosequence, comprising a recently burned scrub during a winter-prescribed fire to scrub where fire did not occur for 40 years. The number of culturable cells was reduced by two orders of magnitude by indirect fire effects and environmental factors, principally hydric stress. However, the duration of fire effects was very short since the microbial community returned to pre-fire numbers and activity by day 47 after fire. Microbial community activity was distinctively related to inoculum density in the soil and litter samples. Soil and litter microbial communities showed differences in metabolic activity. There was no difference in substrate utilization pattern, but there was significant seasonal variation related to the decrease in water content during the month of May. Substrate utilization by litter microbial communities was higher during the month of January compared to soil microbial communities and this relationship was inversed during the month of May probably associated to the more stringent conditions, low water availability, on the litter layer. Seasonal effects outweighed fire effects in this study as this environmental constraint determined the microbial community structure and activity.

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TABLE OF CONTENTS

LIST OF FIGURES.....	vii
LIST OF TABLES.....	x
LIST OF ABBREVIATIONS.....	xi
CHAPTER ONE: INTRODUCTION.....	1
CHAPTER TWO: RESEARCH DESIGN AND METHODOLOGY.....	8
Site description.....	8
Sample collection and laboratory analysis.....	9
Statistical analysis.....	15
CHAPTER THREE: DATA ANALYSIS.....	17
Climate Data.....	17
Fire Temperature Analysis.....	20
Soil Water Content Analysis.....	20
Number of Culturable Cells.....	25
Nutrient Content.....	31
Community Level Physiological Profiling.....	34
CHAPTER FOUR: DISCUSSION.....	41
CHAPTER FIVE: CONCLUSION.....	48
LIST OF REFERENCES.....	50
APPENDIX A CLIMATE DATA.....	56
APPENDIX B C:N REGRESSION ANALYSIS.....	58
APPENDIX C SUBSTRATE UTILIZATION PATTERNS.....	61

LIST OF FIGURES

Figure 1: Happy Creek study site located in Merrit Island National Wildlife Refuge.....	10
Figure 2: Copper tags with fire sensitive paint. Described in (Wally et al 2003).....	13
Figure 3: BD Oxygen Biosensor System microplates (BD Biosciences, Bedford, Massachussets).....	16
Figure 4: Climate data for the sampling season. Data extend from January 5th 2004 to May 31 st 2004.....	18
Figure 5: Prescribed fire at Happy Creek, Merrit Island National Wildlife Refuge (MINWR). March 3, 2004.	19
Figure 6: Maximum fire temperature recorded in 11 and 40 years post-fire plots.....	19
Figure 7: Soil gravimetric water content for pre- and post fire samples for the 11- and 40- year old permanent transects burned during the prescribed fire in March 3, 2004 and long-term study for January and May samplings.....	21
Figure 8: Litter gravimetric water content for pre- and after fire samples for the 11- and 40-year old permanent transects burned during the prescribed fire in March 3, 2004; and long-term study for January and May samplings.....	22
Figure 9: Response of the culturable cell density to fire for soil and litter from 11- and 40-year old transects.....	26
Figure 10: Number of culturable cells soil and litter samples collected in January and May sampling seasons.....	27

Figure 11: Percent of colony formation after 1, 3, 5, 7 and 15 incubation days for soil samples from 0.8-, 2.5-, 6.5-, 11-, and 40-year old transects collected in January and May sampling seasons.....	29
Figure 12: Percent of colony formation after 1, 3, 5, 7 and 15 incubation days for litter samples from 0.8-, 2.5-, 6.5-, 11-, and 40-year old transects collected in January and May sampling seasons.....	30
Figure 13: Carbon and Nitrogen content in dry mass basis in soil and litter samples for the short- term study.....	32
Figure 14: Carbon and Nitrogen content in dry mass basis in soil and litter samples for the long-term study.	33
Figure 15: PCA factor scores for time to peak and peak amplitude in soil and litter samples for the short-term experiment.....	35
Figure 16: PCA factor scores for time to peak and peak amplitude in soil and litter samples for the long-term experiment.....	36
Figure 1-B: Regression analysis for C:N ratio for the 4 scenarios.....	60
Figure 1-C : Soil microbial community time to peak for two substrates glucose and xylan during January and May sampling seasons.....	63
Figure 2-C: Soil microbial community peak amplitude for two substrates glucose and 0.8LE during January and May sampling seasons.....	64
Figure 3-C: Time to peak for different substrates in soil and litter samples for January sampling season.....	65
Figure 4-C: Time to peak for different substrates in soil and litter samples for May sampling season.....	66

Figure 5-C: Time to peak for xylan in soil and litter samples for the short-term experiment.....	67
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LIST OF TABLES

Table 1: Sampling subjects and date, corresponding to the long- and short-term fire effects studies.....	11
Table 2: ANOVA homogenous groups for soil mean gravimetric water content for the short-term study for January and May sampling seasons.....	23
Table 3: ANOVA homogenous groups for soil mean gravimetric water content for the long-term study for January and May sampling seasons.....	23
Table 4: PCA-correlation coefficients of time to peak for soil and litter samples in the short- and long-term experiments.....	38
Table 5: PCA-correlation coefficients of peak amplitude for soil and litter samples in the short- and long-term experiments.....	38
Table A-1: Climatic data for the sampling season and month averages for the decade. Weather data were recorded in the Shuttle Landing Facility, Cape Canaveral.....	58
Table 1-B: Regression analysis parameters for the four studied scenarios.....	61

LIST OF ABBREVIATIONS

PBMS: phosphate-buffered mineral salts.

0.8LE: 0.8 year old transect litter extract.

11LE: 11 years old transect litter extract.

40LE: 40 years old transect litter extract

C: Carbon

N: Nitrogen

% C: Percent Carbon.

% N: Percent Nitrogen.

C:N: Carbon to Nitrogen ratio.

NRFU: Normalized relative fluorescence units.

PCA: Principal component analysis.

CHAPTER ONE: INTRODUCTION

Florida scrub covers 40,000 km² which represents approximately 5 % of the total area in the state of Florida. This ecosystem is valuable as it is home to a high number of endemic species. Many of these scrub species are designated as endangered, threatened, and species of special concern (Christman and Judd 1990).

Even though scrub habitat has been characterized by an insular and discontinuous distribution, the dramatic fragmentation resulting from changes in land use for urban development, agricultural and commercial activities has created a mosaic of scrub surrounded by inadequate habitats for obligate scrub organisms. The scrub ecosystem has suffered an estimated reduction of the 60 % in the aerial coverage from the times of the European settlement (Kautz 1993). One of the main threats to scrub ecosystems besides habitat loss is habitat degradation due to the interruption of the natural cycle of fire caused by anthropogenic influence (Kautz 1993, Menges et al. 1993, Duncan et al. 1999).

Florida scrub is a fire-dependent ecosystem. The regeneration of species and recovery of ecosystem function in scrub is adapted to periodic destruction by fire. Schmalzer and Hinkle (1987) described the distinctive oak-saw palmetto scrub. This type of scrub is dominated by evergreen, sclerophyllous oaks including *Quercus myrtifolia*, *Q. chapmanii*, (Schmalzer and Hinkle 1996), and *Q. geminata*, *Serenoa repens* (saw palmetto), (Schmalzer and Hinkle 1996), and ericaceous shrubs (i.e., *Lyonia fruticosa*, *L. lucida*) lacking a pine canopy. As many fire-adapted plant species that partition a large portion of biomass to the root system scrub oaks accumulate approximately the 87% of the aboveground biomass (Schmalzer and Hinkle 1987), and saw palmetto contains

substantial biomass in its rhizomes as well. Oaks, saw palmettos and the ericaceous shrubs all resprout after fire (Webber 1935, Abrahamson 1984a, 1984b). Oak-saw palmetto scrub is resilient to fire (Abrahamson 1984a, Myers 1990). However, the environmental factors that explain the overall resilience vary along species composition – dominance gradients. Schmalzer and Hinkle (1992) performed an experiment in an oak-saw palmetto scrub which was subject to a prescribed winter fire after a fire suppression period that ranged in between 7 and 11 years. Oak-saw palmetto scrub did not exhibit changes in floristic composition but those stands where the oak was the dominant species, had a higher inertia to fire than saw palmetto dominated or mixed oak-saw palmetto scrub, whereas saw-palmetto dominated areas presented greater elasticity than oak dominated or mixed oak-saw palmetto scrub. Research also demonstrated that species regeneration and consequently recovery of ecosystem function depend on the time between fires and soil moisture (Schmalzer and Hinkle 1996). Among the strategies implemented to maintain the composition and function or restore the Florida scrub ecosystem, besides land acquisition, is the application of prescribed burns or other management techniques like mechanical treatment where fire is unsuitable.

Fire effects over soil biochemistry have been largely studied. Fire is actually an agent of such magnitude that carbon emissions due to fires are estimated to be comparable to fuel emissions (Olson 1981, Crutzen and Andrae 1990, Penner et al. 1992, Mack et al. 1996). Fire has also been demonstrated to produce nitrogen emissions of substantial magnitude (Crutzen and Andrae 1990, Galloway et al. 1995). Trammell et al. (2004) quantified nitrogen losses equivalent to 4.5 years of atmospheric inputs, wet and dry deposition, during a prescribed fire in Oak-Hickory forest of Kentucky. The

temperature of the fire determines the magnitude of nutrient. It is an important biogeochemical process to consider in fire prone ecosystems. Consequently, nutrient loss is highly dependent on the amount of fuel material in the canopy and soil upper layer, the distribution of the fuel material, the soil organic matter (SOM) content and the soil heat conductivity.

Nutrient loss could be explained by different mechanisms such as oxidation-volatilization of components stored in the living biomass, litter layer and subsurface soil (occurring during the fire), conversion to soluble compounds and subsequent leaching through the soil profile and ash transport (both processes occurring post-fire). The C and N in the burned material are basically volatilized as CO₂ and N₂ respectively (Raison et al. 1985). In addition large amounts of sulfur (S) and a smaller amounts of phosphorus (P) (P volatilization occurs at temperatures higher than 500 °C that are most likely to occur during wildfires which present higher temperatures) are volatilized during fire (Pyne et al. 1996). Virtually nothing of Ca, K and Mg pools is lost in the gaseous phase. These minerals and the non-volatilized P are generally the constituents of ash (DeBano and Conrad 1978, Raison et al. 1985, Castelli and Lazzari 2002). However, empirical determinations of volatilization temperatures could produce large underestimations of nutrient loss due to fire. Chemical elements occur mostly as building elements of organic molecules in the standing and dead biomass and soil organic matter, therefore the specific volatilization temperatures for organic associated elements could differ (Raison et al. 1985). Leaching has been demonstrated to be a minor pathway for nutrient loss in comparison to combustion losses (Mackensen et al. 1996, Caldwell et al. 2000, Tremmel et al. 2004). Particle transport (chimney effect) during the fire and post-fire ash

convection are not major pathways for nutrient loss during and after moderate fires, whereas increments in fire temperature could produce not only significant nutrient transport (DeBano et al. 1998, Johansen 2001) but also long- distance transport of plant material (Pisaric 2002) which could have ecological repercussions. Bio-transportation (ant mounding) of ash has also proven to be a significant process in some environments (Andersen and Yen 1985, Dragovich and Morris 2002), up to 36% of the total ash produced, particularly after moderate fires. Regarding pH changes, Schmalzer and Hinkle (1991) found a modest increase in soil pH after fire that lasted approximately for one year. In addition, an increase in calcium was found after six months, but this had disappeared after one year after the fire. The data collected in this study suggested the occurrence of vertical transport of ash material which has a higher exchange and adsorption capacity than the native soil.

The above mentioned physical and biochemical changes in the soil may be determining factors for plant regeneration and ecosystem function probably associated with the effects of fire over the soil microbial community. Fire has direct and indirect effects over the soil microbial community. The increase on the superficial soil temperature has been demonstrated to have a temporary sterilizing effect on the soil (DeBano et al 1998). Indirect effects would result from the effect of fire on physical and chemical characteristics of the soil. Previous studies (Henrot and Robertson 1994, Díaz-Ravina et al. 1996, Dumontet et al. 1996, Ross et al. 1997) have suggested that microbial activity in the upper horizon may be impeded in the long term by the effects of strong regular fires due to accelerated desiccation of the bare surface during the dry season, in addition to the reduction of organic substrates consumed by the fires. This would

particularly affect the heterotrophic and saprophytic bacteria, and even more those microorganisms inhabiting the litter layer and superficial soil.

Soil microbial communities regulate ecosystem processes such as decomposition, nutrient cycling, and soil carbon storage (Vitousek and Matson 1985, Zak 1994, Bohlen et al. 2001, Reynolds et al. 2003). They also enhance metabolic activities that result in plant pathogen suppression (Milus and Rothrock 1993, Mills and Bever 1998, Van Elsas et al. 2002, Molina et al. 2003), sometimes stimulated by components of the plant community itself (Hebbar 1991, Brown et al. 1997). In the event of fire, microbial communities may act as a buffer, preventing nutrients to leach out from the system (Singh et al 1989, 1991). Microbial immobilization and further cell death would make these nutrients available in moderate doses; therefore this mechanism would be fundamental for nutrient conservation in oligotrophic ecosystems. However, the impact of soil microbial communities on ecosystem regeneration and recovery of function after fire disturbance remain to be understood.

Studies of scrub ecosystem have focused on plant species resurgence (Schmalzer and Hinckle 1987, 1992, 1996, Schmalzer 2003, Menges and Quintana-Ascencio 2004), plant community composition, and recovery (Hawkes and Menges 1995, 1996, Menges and Kimmich 1996, Menges and Hawkes 1998, , Quintana-Ascencio and Morales 1997, Quintana-Ascencio and Menges 2000, Quintana-Ascencio et al. 1998, 2003) and animal habitat requirements (Breininger 1995, Duncan 1995) to assess the reestablishment of ecosystem function. Currently, the sparse knowledge of the scrub soil microbial community is restricted to fungi and cyanobacterial crusts (Hawkes and Fletchner 2002). There are no studies concerning the soil bacterial community, their interactions with plant

community, or their dynamics after fire disturbance. Determining how the bacterial fraction of the microbial community is affected by fire and consequent soil physical and chemical disturbance, will help define its role during scrub regeneration. The question are how is microbial function affected by fire disturbance and what are the repercussions on their interaction with the plant community? The aim of this study is to determine fire effects upon soil microbial communities in terms of microbial abundance and physiological profiling thorough a chronosequence study. This study also comprises the study of seasonal effects with sampling extended over a period of four months, capturing significant temperature and rainfall variations.

The hypotheses addressed in this study are:

- Fire will have a direct effect upon the soil microbial community observable as a reduction in the number of cells. This will be evaluated through direct counts.
- There will be a shift in microbial community structure throughout the secondary succession gradient. This would be appreciable as an increase on the percent of fast growing organisms versus slow growing organisms (DeLeij FAAM et al. 1993, Garland et al. 2001) right after fire. It is also expected a gradual decrease of fast growing microorganisms with increasing time after fire. Differences in microbial community physiological profile along the chronosequence, associated with changes in the available substrates for the community to act upon are expected. This would result in higher utilization of simple substrates after-fire,

associated to relatively more abundant of colonizer, generalist, or r-type of organisms after the disturbance.

- Changes in substrate quality are expected due to a differential effect of fire over the carbon and nitrogen pool. Fire is expected to reduce the total C and N pool, which will recover with time after fire.
- There will be changes in metabolic status associated with fire and consequent desiccation and the action of stressing factors or stringent environmental conditions; this will result in a reduced microbial community and lower metabolic activity.

This study will generate knowledge on fire-induce microbial community shifts that will be applicable to evaluate the effects on other components of the ecosystem, and potentially generate guidelines to improve fire management practices.

CHAPTER TWO: RESEARCH DESIGN AND METHODOLOGY

Site description

Merritt Island National Wildlife Refuge (MINWR) contains 57,000 ha of scrub community. This area was acquired in different portions by the National Aeronautics and Space Administration since 1962. Currently the U.S. Fish and Wildlife Service manages the refuge. The climate in the area is subtropical, warm and humid. The 100-yr mean annual precipitation is 131 cm, with high annual variability. One-hundred-year mean maximum and minimum temperatures in July, the hottest month, are 33.38 °C and 21.88 °C, respectively, and 22.38 °C and 9.58 °C, in January, the coldest month (Mailander 1990). Thunderstorms and lightning strikes are common in the summer season (Eastern Space and Missile Center 1989)

The study site, Happy Creek, is located in MINWR (28° 38'N, 80° 42' W). The soils in the area are Pomello (Arenic Haplahumods) and Paola sands (Spodic Quartzipsamments). Both soils are well-drained, acidic, and low in nutrients. These soils have an O horizon, constituted of undecomposed litter material. The highest nutrient concentrations are found in the O and A horizon as a product of the slow decomposition and nutrient lixiviation (Schmalzer and Hinkle 1996). The vegetation is dominated by two oak species, *Quercus myrtifolia* and *Q. geminata* constituting up to 87% of aboveground biomass in this system (Schmalzer and Hinkle 1996). Also abundant is saw palmetto, *Serenoa repens*, which typically contains considerable biomass in its rhizomes (Schmalzer and Hinkle 1996). Minor species included *Q. chapmannii*, *Lyonia fruticosa*, *L. lucida*, *Vaccinium myrsinites*, and *Galactia eliotii* (Dijkstra et al. 2002). The

experimental site is representative of a fire-maintained, scrub oak palmetto community described in Breininger and Schmalzer (1990) and Schmalzer and Hinkle (1996).

Sample collection and laboratory analysis

Samples were collected from 15-m line-intercept permanent transects established in 1983 that have been used in past studies (Schmalzer and Hinkle 1992, Breininger et al. 1994, Schmalzer 2003). Changes in the microbial community were studied along four stands, burned in prescribed fires 40 years (R25, R26 and R28), 11 years (R20, R21 and R22), 6.5 years (P1, P2 and P6), 2.5 years (P13, P14, and P15), and 10 months (P3, P4 and P5), prior to the beginning of the experiment, representing different fire suppression periods. The 11-year plot was mechanically treated 5 years ago. Samples from all transects were initially collected in January (winter season) (see Table 1), prior to the prescribed fire and also in the unburned transects in the month of May (spring season) to study long-term effects and seasonal effects of fire.

Fire occurred on March 3rd. The 11- and 40-year post-fire plots were burned and used to record the immediate and short term effects of fire. Samples were collected from 11 and 40 years plots in days 2, 10, 26, 47 and 69. These transects were also the subject of fire temperature studies. Maximum fire temperature was measured utilizing pyrometers (Figure 2), consisting of copper tags painted with fire sensitive paint covering a range from 79.4 °C to 871 °C, at approximately 10°C intervals (Wally et al 2003). Thirty pyrometers were distributed every meter with a separation of 1 m to each side perpendicular to the transect.

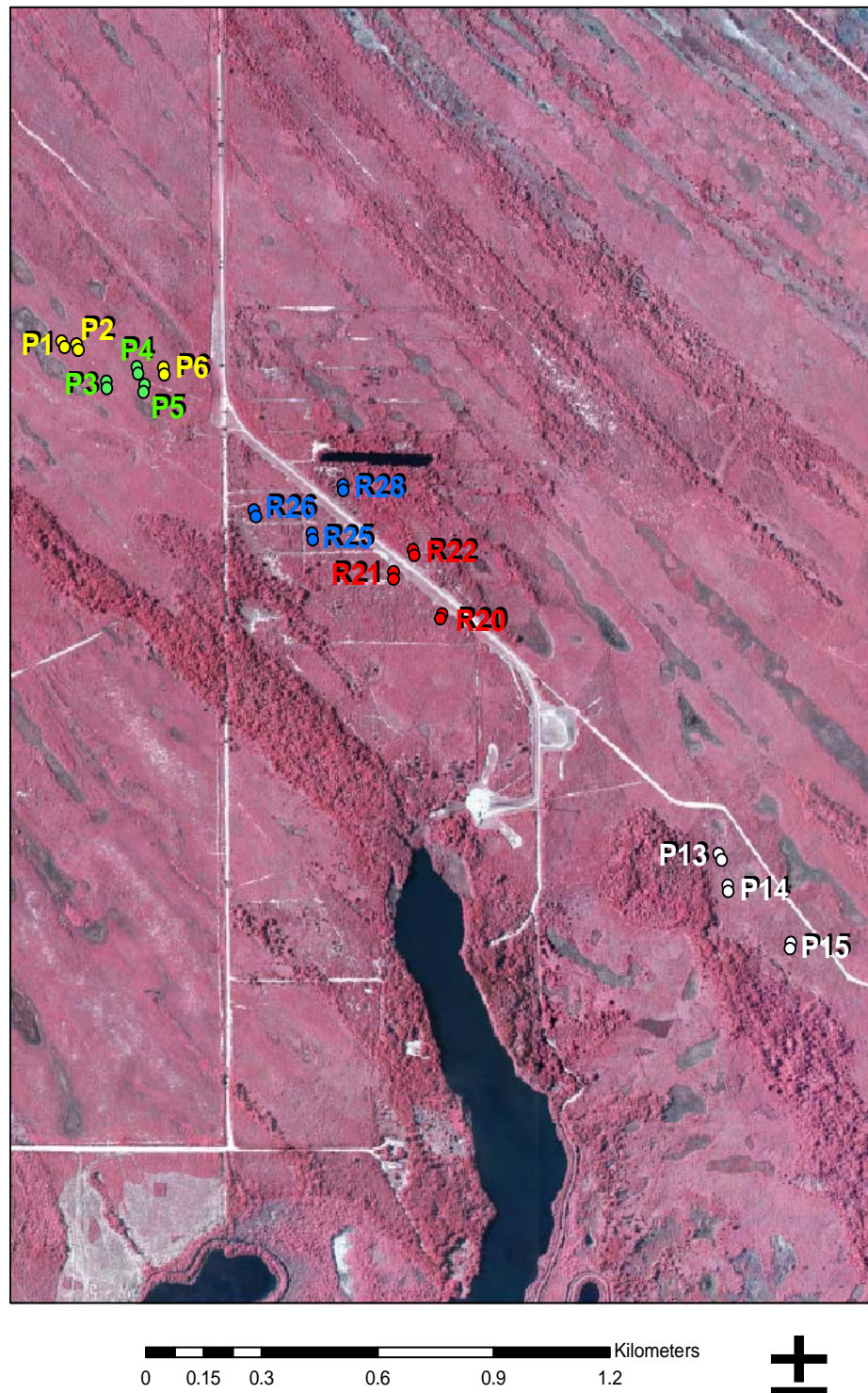


Figure 1: Happy Creek study site located in Merritt Island National Wildlife Refuge.

Permanent transects are colored by time to last fire event: 10 months (green).

2.5 years (white), 6.5 years (green), 11 years (red), and 40 years (blue).

Table 1: Sampling subjects and date, corresponding to the long and short-term fire effects studies.

<i>Study</i>	<i>Sampling date</i>	<i>Transects</i>	<i>Sample Size</i> <i>(x samples x y transects)</i>
Long term	26 th January 2004	All transects	n = (3 x 3) x 5
	18 th May 2004	P3, P4 and P5.	n = (3 x 3)
		P1, P2 and P6.	n = (3 x 3)
	25 th May 2004	P13, P14 and P15.	n = (3 x 3)
		R22 and R28	n = (2 x 2)
		R26(unburned section)	n = (1 x 1)
Short term	5 th March 2004	R20 and R21 R25 and R26	n = (1 x 2)
	15 th March 2004		n = (2 x 4)
	29 th March 2004		n = (2 x 4)
	19 th April 2004		n = (2 x 4)
	11 th May 2004		n = (2 x 4)

The pyrometers were collected at the first post-fire sampling. Maximum fire temperature corresponded to the maximum melting temperature of the paint, among the melted fire sensitive paints.

Leaf litter was removed and collected from 15 points located along the three 15 m transects for each fire suppression period, leaving bare ground from which soil (0-10 cm deep) samples were extracted. The fifteen sub-samples of soil and litter were separately homogenized into two samples of each type per transect.

Laboratory analyses were be completed in the Space Life Sciences Laboratory at Kennedy Space Center.

Sample conditioning

Soil and litter lecheates were prepared with deionized water in 1:2.5 and 1:5 dilutions respectively. Samples were blended for 1 minute and allowed to precipitate for 2 minutes. Lecheates were filtered through a 30 mesh sieve to remove the coarse particles.

Culturable cells counts

Soil and litter lecheates were serially diluted and plated in concentrations that ranged from full strength to 10^{-5} . Afterwards these suspensions were used to inoculate 100 μ l aliquots onto R2A agar plates to obtain culturable cells numbers. Plates were incubated at room temperature (approximately 25 °C). Microbial colonies were counted on day 1, 3, 5, 7 and 15 after the inoculation to obtain the rate of colony formation over time.

Community level physiological profiling

Substrate utilization kinetic analysis was performed using BD Oxygen Biosensor System microplates (BD Biosciences, Bedford, Massachussets) (Figure 3). Diverse substrates were prepared: CMC, chitin and xylan at 750 mg liter⁻¹ concentration, mannose and glucose at 270 mg liter⁻¹ concentration, cas aminoacids 900 mg liter⁻¹, inositol 0.1 mM, citrate 0.3 mM and xylose 1.5 M. CMC, chitin and xylan solutions were autoclaved for x hours at x °C, the remaining substrates were filter-sterilized with 40 nm pore diameter cellulose filters.



Figure 2: Pyrometers consisting of copper tags painted with fire sensitive paint.
Described in (Wally et al 2003).

Additionally, three litter extracts at 1:5 concentration, corresponding to the 0.8 year (0.8LE), 11 year (11LE) and 40 year (40LE) post- fire plots, were prepared for use as substrates. The litter material was heated for 3 hours to 46 °C and filtered- sterilized. Deionized water was used as blank substrate.

Fifty microliter (µl) of sample were suspended in 50 µl sterile phosphate-buffered mineral salts (PBMS) (7 g of K_2HPO_4 liter⁻¹, 3 g of KH_2PO_4 liter⁻¹, 0.1 g of $MgSO_4$ liter⁻¹, 0.5 g of $[NH_4]_2SO_4$ liter⁻¹, 0.01 g of $CaCl_2$ liter⁻¹, 0.005 g of $FeSO_4$ liter⁻¹, 0.0025 g of $MnSO_4$ liter⁻¹, and 0.0025 g of Na_2MoO_4 liter⁻¹) and inoculated into the wells with 50 µl of substrate or deionized water. Plates were read in a Dynex MFX and Victor® Microplate Fluorometer at 485 nm excitation and 604 nm emission wavelengths with the top reading mode for a period of 48 hours. After fire samples were inoculated in replicate plates and read simultaneously in both readers.

Soil and leaf litter chemical analysis

Soil and litter samples were dried in a drying oven at 70 °C to constant weight. Gravimetric water content (GWC) was calculated based on the gravimetric difference of the moist and dry sample. Dry samples were ground and used for substrate quality analysis. Substrate quality was based on percent carbon (%C) and nitrogen (%N) and the relationship between them, carbon to nitrogen ratio (C: N). This analysis was performed using a Perkin Elmer 2400 Series II CHNS/O analyzer.

Statistical analysis

Statistical significance on mean differences among water content, substrate quality and colony forming units data were analyzed performing ANOVA (significance level $p = 0.05$) (SPSS version 11.0).

Substrate utilization kinetic data was based on normalized data. Fluorescence readings were divided by the fluorescence measurements at the one-hour time, obtaining normalized relative fluorescent units (NRFU). Data were summarized by two parameters, Peak amplitude and time to peak. Peak amplitude corresponded with the maximum NRFU and time to peak corresponded to the time at which the maximum NRFU occurred. These two parameters have shown to be suitable parameters for sample comparison (Garland et al. 2003). Data from both readers were analyzed with a paired t-test (SPSS version 11.0). Substrate utilization data which revealed significant differences were disregarded for further analysis. Variability in substrate utilization kinetics was analyzed by principal component analysis (PCA-Correlation matrix) (SPSS version 11.0).

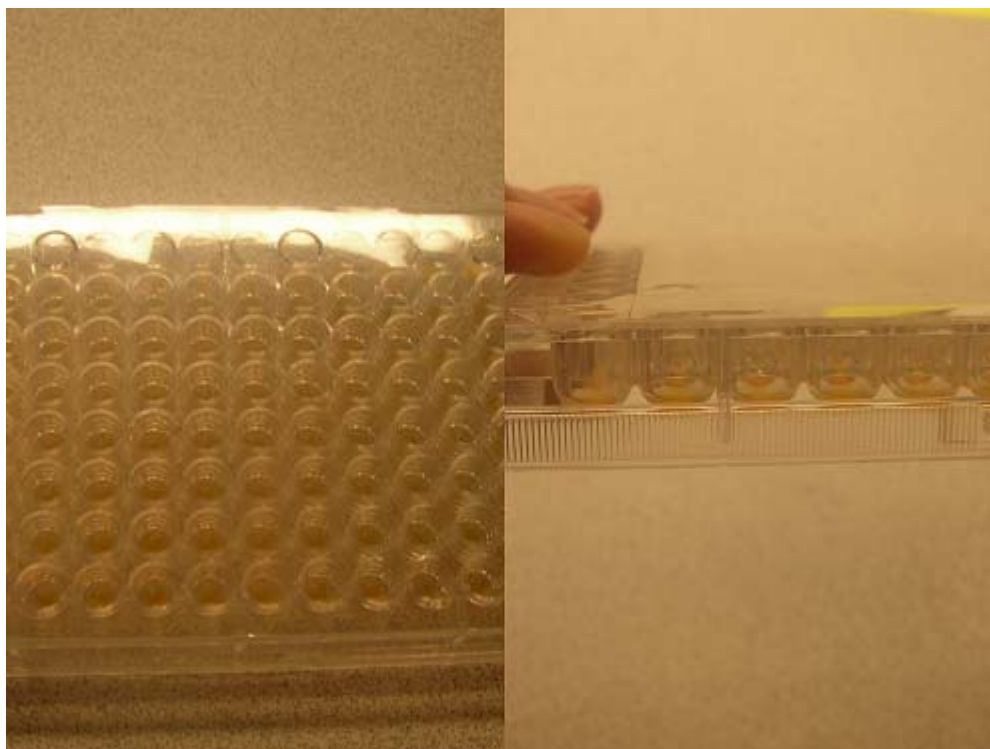


Figure 3: BD Oxygen Biosensor System microplates (BD Biosciences, Bedford, Massachusetts).

CHAPTER THREE - RESULTS

Climate Data

The sampling season extended from January 26 to May 25. Temperature values did not deviate from the expected for the area and season (*Appendix 1- Climate data*). Monthly temperature averages during the study was 2 °C below the decadal monthly average. The relative humidity presented similar values to the decadal monthly averages, except for February, when relative humidity was 87 % and the decadal average was 76 %. Precipitation data for the sampling season presented a scattered distribution, with very rainy and very dry periods (Figure 4). During the weeks from 19th January to February 9th 2004, in which occurred the first sampling, the total precipitation was 78.49 mm. This amount of precipitation even exceeds the decadal total maximum precipitation for either month. During the month of February, prior to the prescribed fire, the total precipitation was higher than the total average for the decade (Table 1). The total precipitation values for the month of February were 88.14 mm; the decadal the average was 66.04 mm.

Contrarily, March, April and May 2004 were drier than normal. During March 2004, the total precipitation was 16.56 mm; the average precipitation for the decade was 88.90 mm. The total precipitation for April was 30.73 mm; the average for the decade was 70.10 mm. May, when the last samples were collected, also had lower precipitation than the decadal average, the total precipitation was 48.26 mm; the decadal average was 74.48 mm.

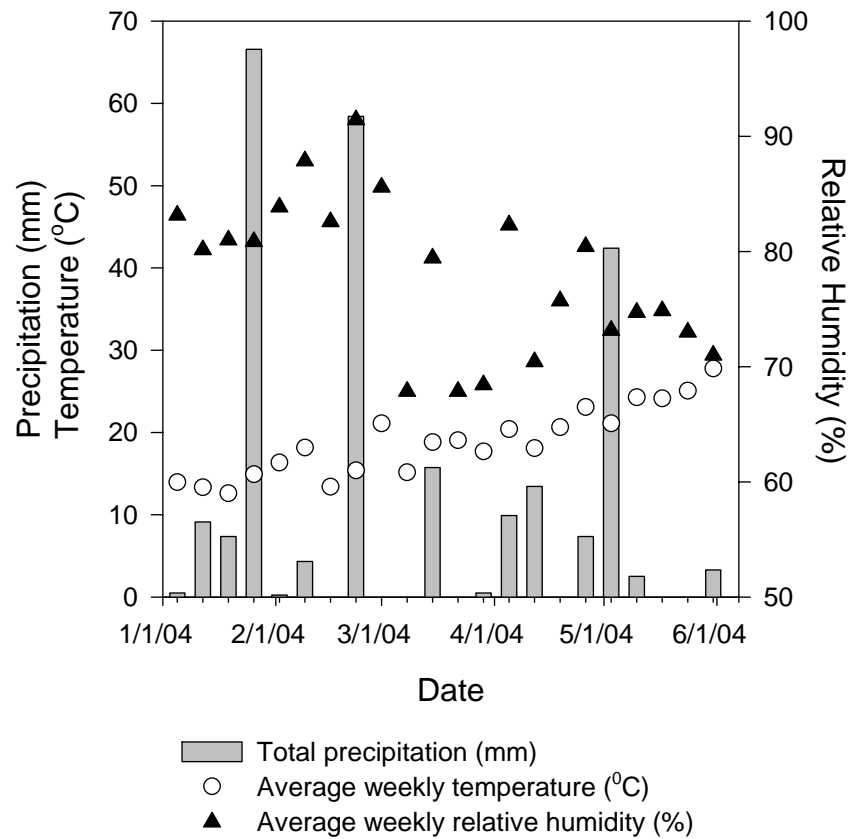


Figure 4: Climate data for the sampling season. Data from January 5th 2004 to May 31st 2004. Precipitation bars represent cumulative precipitation for weekly periods. Weather data were recorded at the Shuttle Landing Facility, Cape Canaveral.



Figure 5: Prescribed fire at Happy Creek, Merritt Island National Wildlife Refuge (MINWR). March 3, 2004.

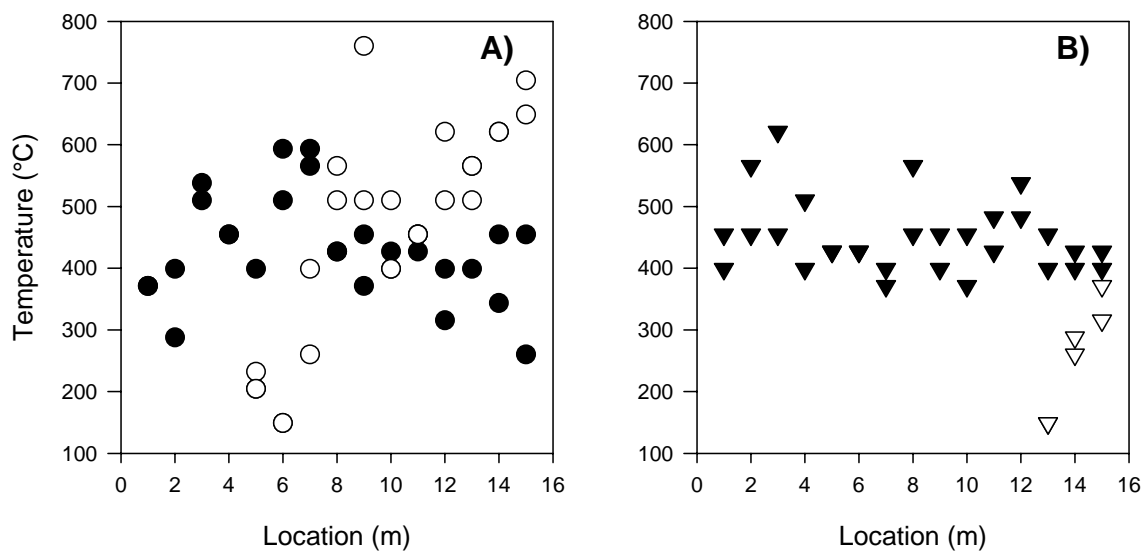


Figure 6: Maximum fire temperatures for the A) 11 year post fire transects (● R20 and ○ R21) and B) 40 year after fire (▼ R25 and ▽R26) permanent transects burned during the prescribed fire in March 3, 2004.

Fire Temperature Analysis

The prescribed burning in the study area occurred quickly in each location as shown in Figure 5. The maximum fire temperature recorded in the field was different for all transects. The 11-year transects exhibited spatially heterogeneous maximum temperatures. Transect R20 maximum temperatures ranged from 204 – 565 °C. Transect R21 maximum temperatures ranged from 148-760 °C. The 40-year old transect, R25 had a maximum temperature that ranged between 398 and 621 °C which was consistent maximum temperatures during the prescribed fire. Transect R26 burned partially; only three meters corresponding to the locations 12 to 15m were burned and the temperatures ranged from 148-315 °C.

Soil Water Content Analysis

Pre and post-fire water content was not significantly different (Paired t-test) between the 11 and 40 years old transects (Figure 7-A). Immediately after fire samples, corresponding to day 2 after fire revealed significant differences in water content with the pre-fire samples content for the 11-year old transect, possessing a higher water content (Figure 4-A) post fire (ANOVA, $p=0.05$). Even though the fire should produce a higher evaporation in the superficial litter and soil layers, the occurrence of a dry period right before the sampling and precipitation after fire (Figure 4) explains the increase in water content. The soil water content was more variable in the 11-year old transects, yielding a wider range of GWC (Table 2) compared to the 40-year transects.

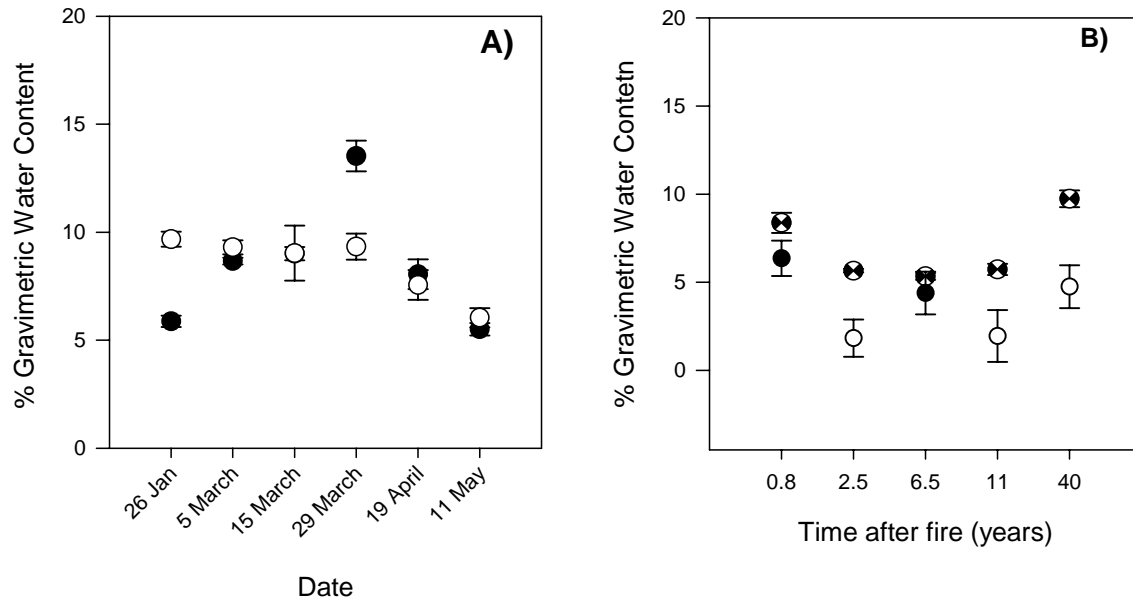


Figure 7: Soil gravimetric water content for A) Pre- and post fire samples for the 11 years (●- R20 and R21) and 40 year (○- R25 and R26) old permanent transects burned during the prescribed fire in March 3, 2004 and B) long-term study for January (●) and May samplings. May samplings were collected on May 18th (●) R20 and May 25th (○), bars indicate the standard deviation.

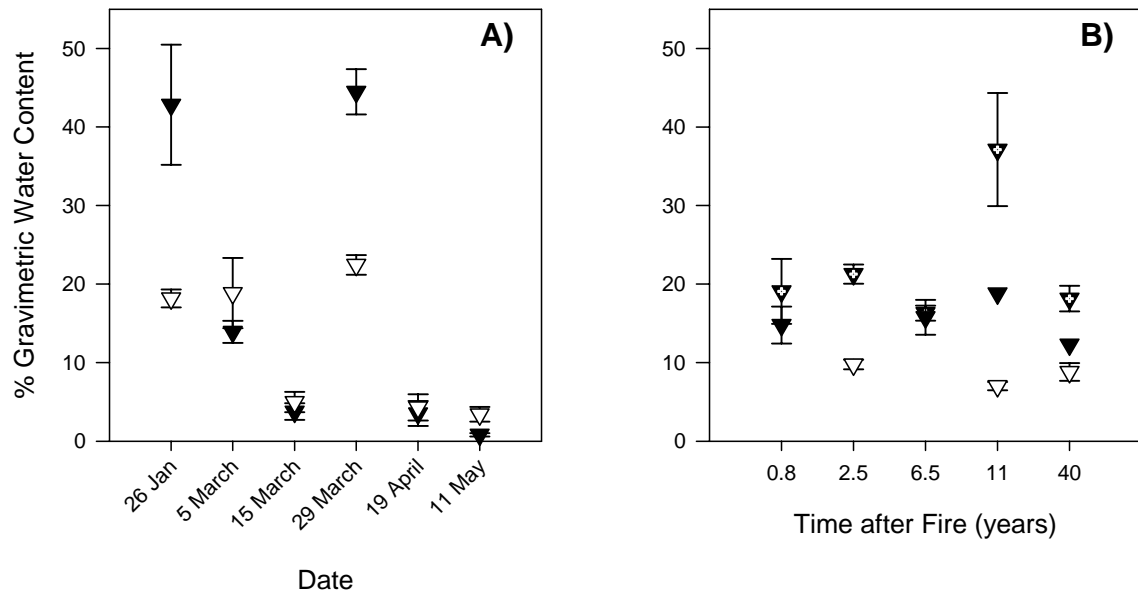


Figure 8: Litter gravimetric water content for A) Pre- and after fire samples for the 11 years (▼ - R20 and R21) and 40 year (▽ - R25 and R26) old permanent transects burned during the prescribed fire in March 3, 2004; and B) long-term study for January (▽) and May samplings. May samplings were collected on May 18th (▼) R20 and May 25th (▽); bars indicate standard deviation.

Table 2: ANOVA homogenous groups for soil mean gravimetric water content for the short-term study for January and May sampling seasons ($p=0.05$).

Date	Day	11 years	40 years
Jan 26 th	Pre-fire	5.87 ^c	9.67 ^d
March 5 th	3	8.66 ^b	9.30 ^d
March 15 th	13	9.00 ^b	9.03 ^{de}
March 29 th	27	13.52 ^a	9.33 ^d
April 19 th	49	8.05 ^b	7.55 ^{de}
May 11 th	79	5.50 ^c	6.04 ^e

The soil water was not as variable as the litter water content. The soil presented in general lower water contents than the litter and these values were less variable. The litter presented a large range of water content with very low water contents, approximately 5 % during the drought high water content after a rain event that ranged from 18 to 45 %.

The soil water content presented significant differences for the January and May sampling seasons in the 2.5-, 11- and 40- year old FSP transects (ANOVA, $p=0.05$), being the first ones the driest among all the transects during May sampling season (Figure 4-B). In all cases, January soil GWC was higher than May GWC. The 6.5 year transects did not exhibit differences between seasons, its initial water content was the lowest among the transects and the final water content was one of the lowest with a mean of 5.34 and 4.39 percent correspondingly (Table 3).

Table 3: ANOVA homogenous groups for soil mean gravimetric water content for the long-term study for January and May sampling seasons ($p=0.05$).

Time after fire (years)	January	May
0.8	8.37 ab	6.36 bc
2.5	5.66 bc	1.83 d
6.5	5.34 c	4.39 cd
11	5.72 bc	1.95 d
40	9.73 a	4.75 c

Pre and post fire litter samples did not show significant differences between the 11- and 40-year old samples, since there was not consistent trend in water content along the study for none of the transects. However, the 11-year old transects presented a wider range in water content and also a larger variability (larger standard deviation) (Figure 5-A). Litter samples did not present significant differences between the January and May sampling seasons (Paired t-test). This would be explained by the occurrence of precipitation between the 18th and 25th of May which increased the variability between and among the samples (Figure 5-B). It is remarkable that litter samples collected on May 25th had water content in the range of the January samples.

Number of Culturable Cells

The number of culturable cells was higher in the litter than in the soil for the short- and long-term studies. The short-term fire effects were moderate at first, in the soil and litter samples. However, there was a more pronounced reduction in the number of culturable cells in the 40-year old transects compared to the 11-year old transects. By March 15th (12 days after fire), there was a drop of three orders of magnitude in the number of culturable cells in the soil. However this reduction was of very short duration, by the 16th of April (49 days after fire) the number of culturable cells had surpassed that of the pre-fire samplings (Figure 9-A). This reduction was not observed in the litter layer since the samples collected between day 2 and 49 were discarded (Figure 9-B).

The number of cells in the soil and litter in the long-term experiment, did not differ after the 10-month post-fire. January cell counts were higher for all five scenarios in both substrates indicating possible seasonal effects. The largest cell count differences in soil were found in the 0.8, 2.5 and 6.5 years after fire whereas the older transects 11 and 40 year presented the smallest differences in cell counts between sampling seasons (Figure 10-A). In the 40-year old transects, the differences between January and May sampling were the smallest. Litter cell counts were smaller between sampling seasons compared to the soil samples and contrarily to the soil samples, the older transects presented the largest seasonal variation (Figure 10-A & B).

Colony formation presented different patterns for the January and May sampling seasons for both soil and litter samples. Soil colony formation from samples collected in January showed a slower colony formation for the older transects, than the younger ones

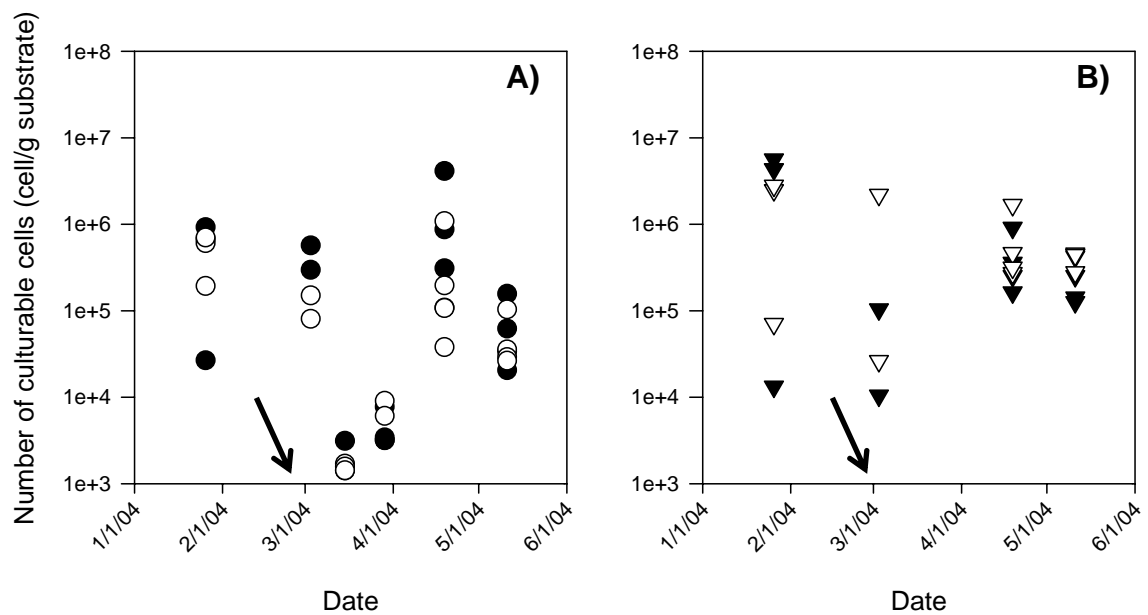


Figure 9: Response of the culturable cell density to fire (after 15 days of incubation at 25 °C); for A) Soil from 11 year (●) and 40 year (○) post-fire transects; and B) Litter from 11 years (▼) and 40 years (▽) post-fire transects.

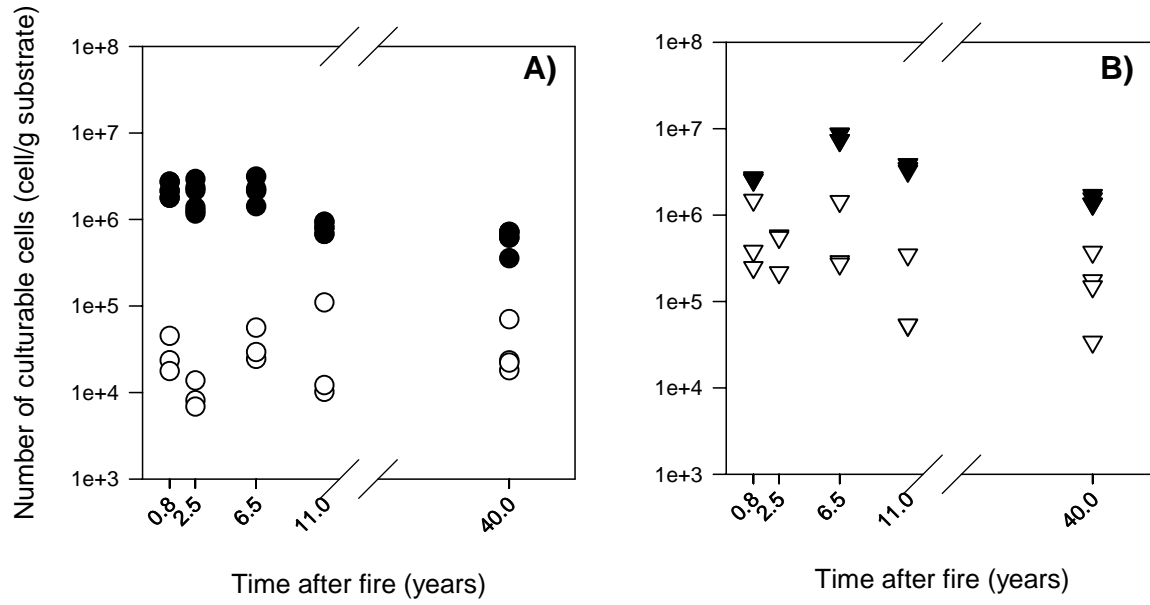


Figure 10: Number of culturable cells (after 15 days of incubation at 25 °C); for 0.8, 2.5, 6.5, 11 and 40 years after fire for A) Soil samples collected in January (●) and May(○)sampling seasons, and B) Litter samples collected in January and (▼) May(▽) sampling seasons.

which showed 50 % of total colony formation by day 1 (Figure 11). The older transects, 11 and 40 years FSP showed 20-30 % of colony emergence by day 1 for the January sampling (Figure 11- D & E) whereas the younger transects showed 40 % colony emergence by day 1 (Figure 11- A, B & C). In the case of samples collected in May, approximately 60 % of the colonies emerged for all the transects except for the 6.5 year FSP transects which showed 40 % colony emergence by day 1. (Figure 11-C). By day 3 almost all of the colonies developed for both January and May samples (Figures 11 & 12). January litter samples showed a similar pattern to the soil in terms of colony formation, the older transects presented the smallest number of colonies by day one and the values were similar to those of the soil samples, 20-25% (Figure 12). The younger transects showed a faster formation of colonies with 58 and 65 % of the colonies formed by day 1. May litter samples showed as well a similar the trend to the corresponding soil samples: colony emergence was higher excepting in the 2.5- and 6.5-year old transects (Figure 2- B & C –Appendix 2). Litter samples showed a longer period to complete the colony formation compared to soil samples, since until day 7 differences in percent of emerged colonies were evident among the samples (Figures 11 & 12). Even though there were not statistical differences, the consistent trend among the different transects, substrates and seasons could indicate the relevance of this analysis. Colony formation did not show a pattern for the short term study in either soil or litter samples.

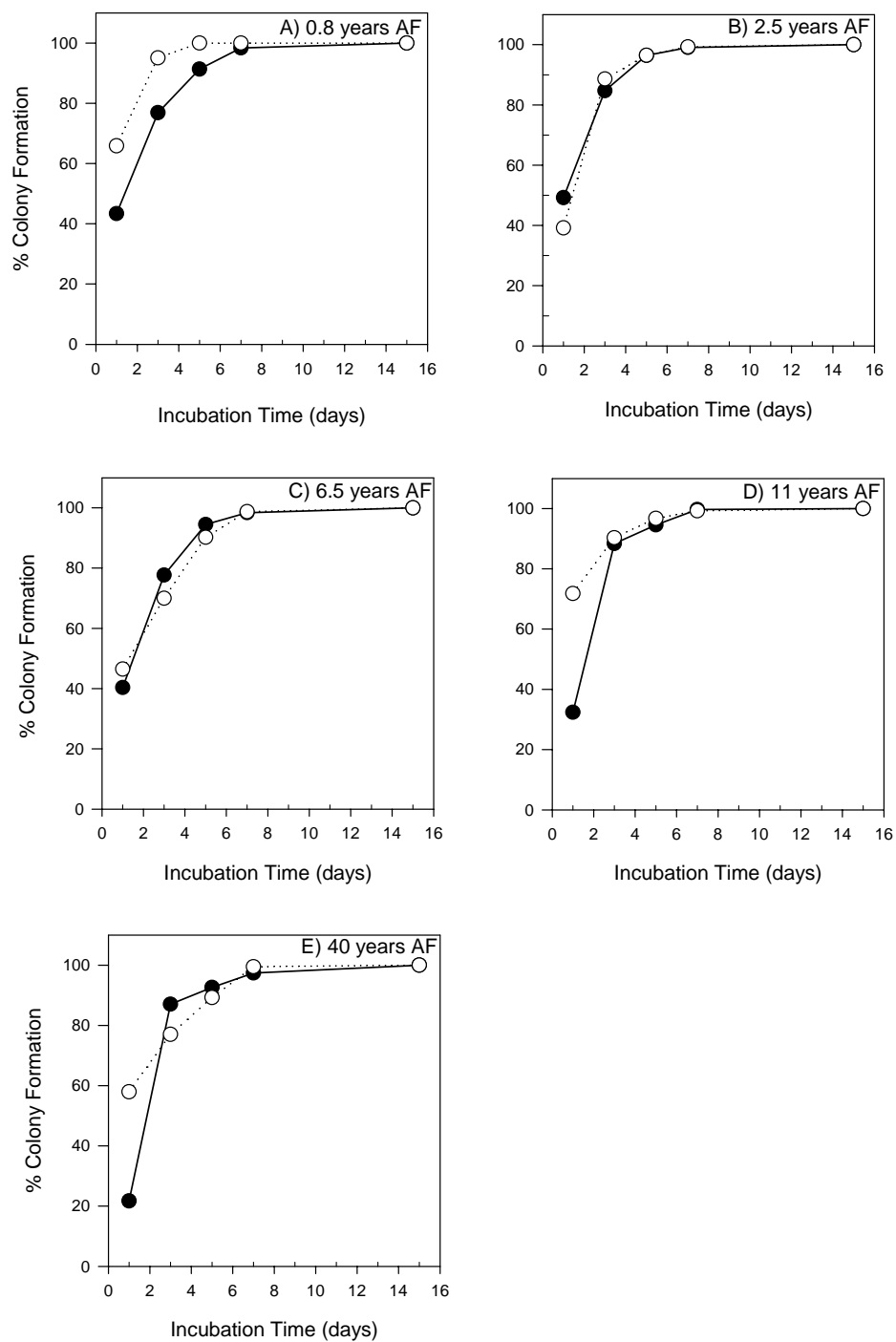


Figure 11: Percent of colony formation after 1, 3, 5, 7 and 15 incubation days at 25°C for A) 0.8, B) 2.5, C) 6.5, D) 11 and E) 40 years FSP of soil samples collected in January (●) and May (○) sampling seasons.

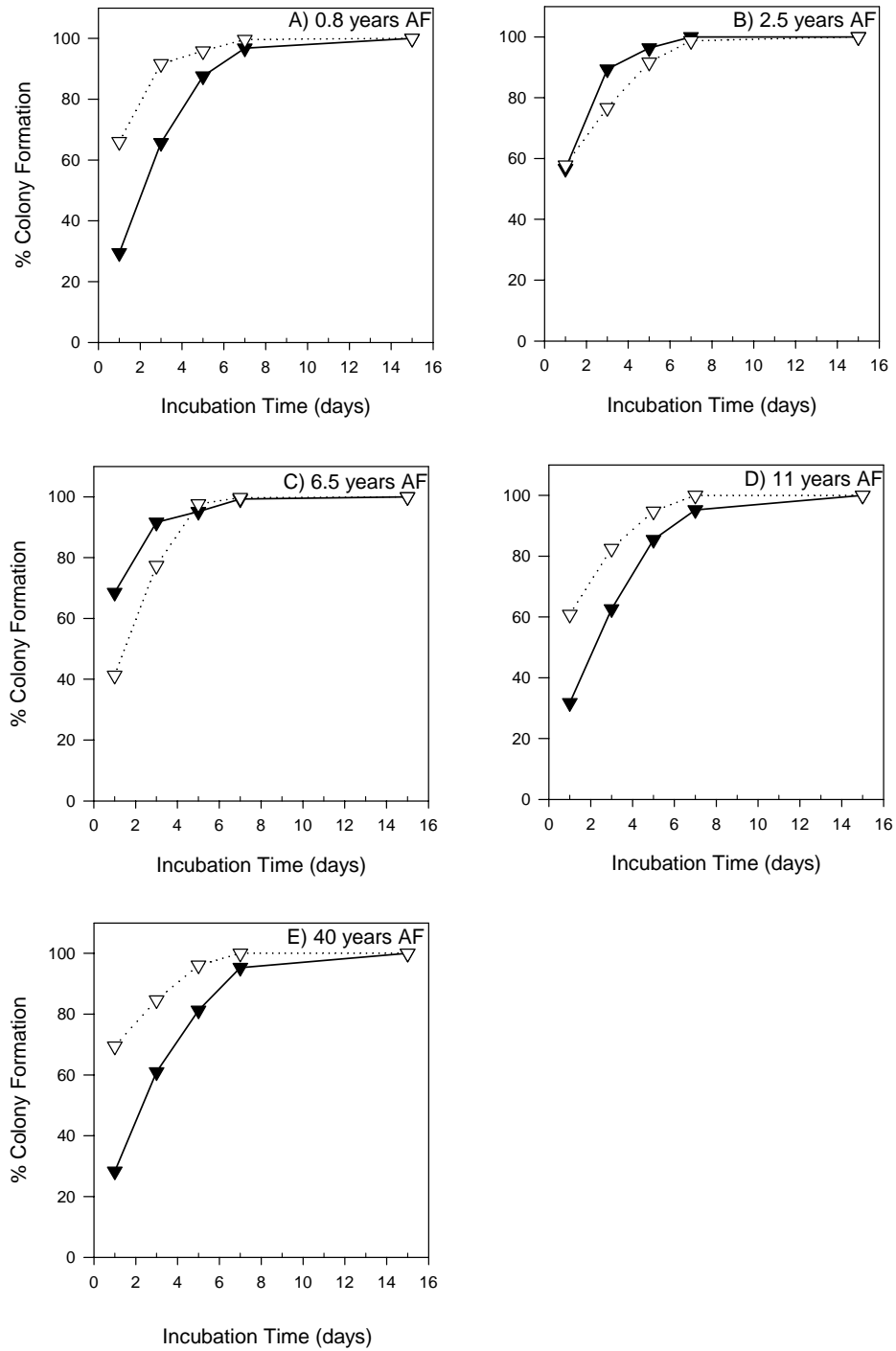


Figure 12: Percent of colony formation after 1, 3, 5, 7 and 15 incubation days at 25°C for A) 0.8, B) 2.5, C) 6.5, D) 11 and E) 40 years FSP of litter samples collected in January (▼) and May (▽) sampling seasons.

Nutrient Content

Fire produced a significant decrease in carbon and nitrogen content among the 40-year old transects (Figure 13). By day 13, the values seemed to have returned to the pre-fire nutrient contents. A remarkable fact is that within the short term experiment, decreases in the nutrient content on the litter layer, seemed to be related to soil increases in that particular content, particularly on the 29th of March there was an increase in the C content in the soil and a decrease in the litter (Figure 13-A and C).

Carbon to Nitrogen linear correlations, C:N ratios (Figure 1-Appendix 2) showed no difference in this parameter between the two fractions, meaning that carbon and nitrogen accumulated at the same rate in litter and soil, and that via leaching, the transfer of carbon and nitrogen from the litter layer to the soil was proportional. The results of the paired t-test did not showed significant differences between the January and the May sampling in soil C (Table 1- Appendix 2). There were no significant seasonal differences for soil N content either. The soil C content did not differ consistently (ANOVA, $p < 0.05$) among the five FSP, showing an erratic pattern probably related to larger site variations than fire effects. The C:N correlation analysis showed differences in C:N ratio in the long term for the soil samples. The values were lower, however conclusions on carbon leaching or higher decomposition and consequent higher concentrations in the organic matter cannot be inferred since the parameter analyzed was Total C and Total N, with no fractionation of these pools.

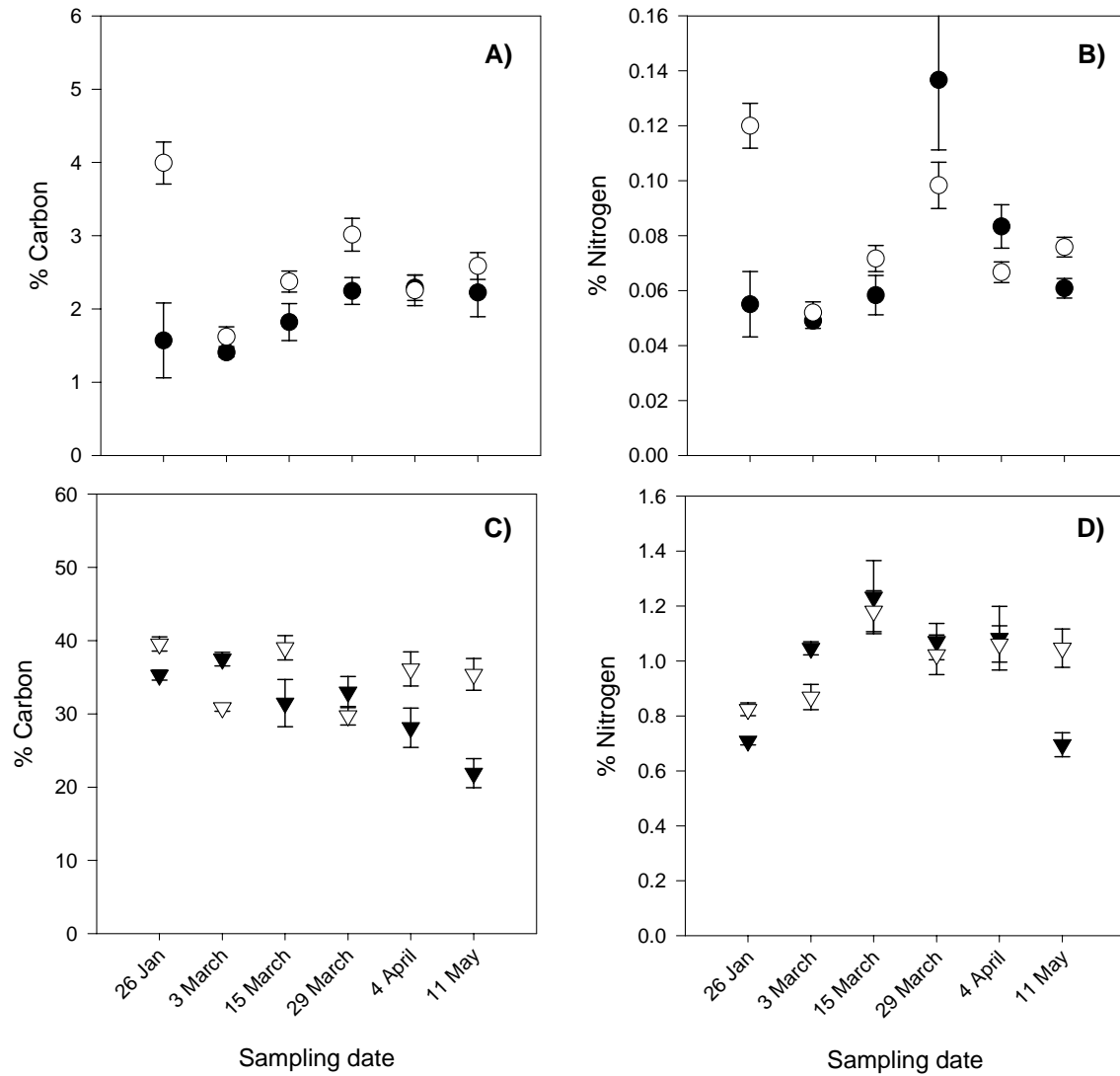


Figure 13: Carbon (A and C) and Nitrogen (B and D) content in dry mass basis for soil (●,○) and litter (▼,▽) for the short-term study. Closed signs correspond to the 11 year transects and open signs correspond to the 40 year transects; bars indicate standard deviation.

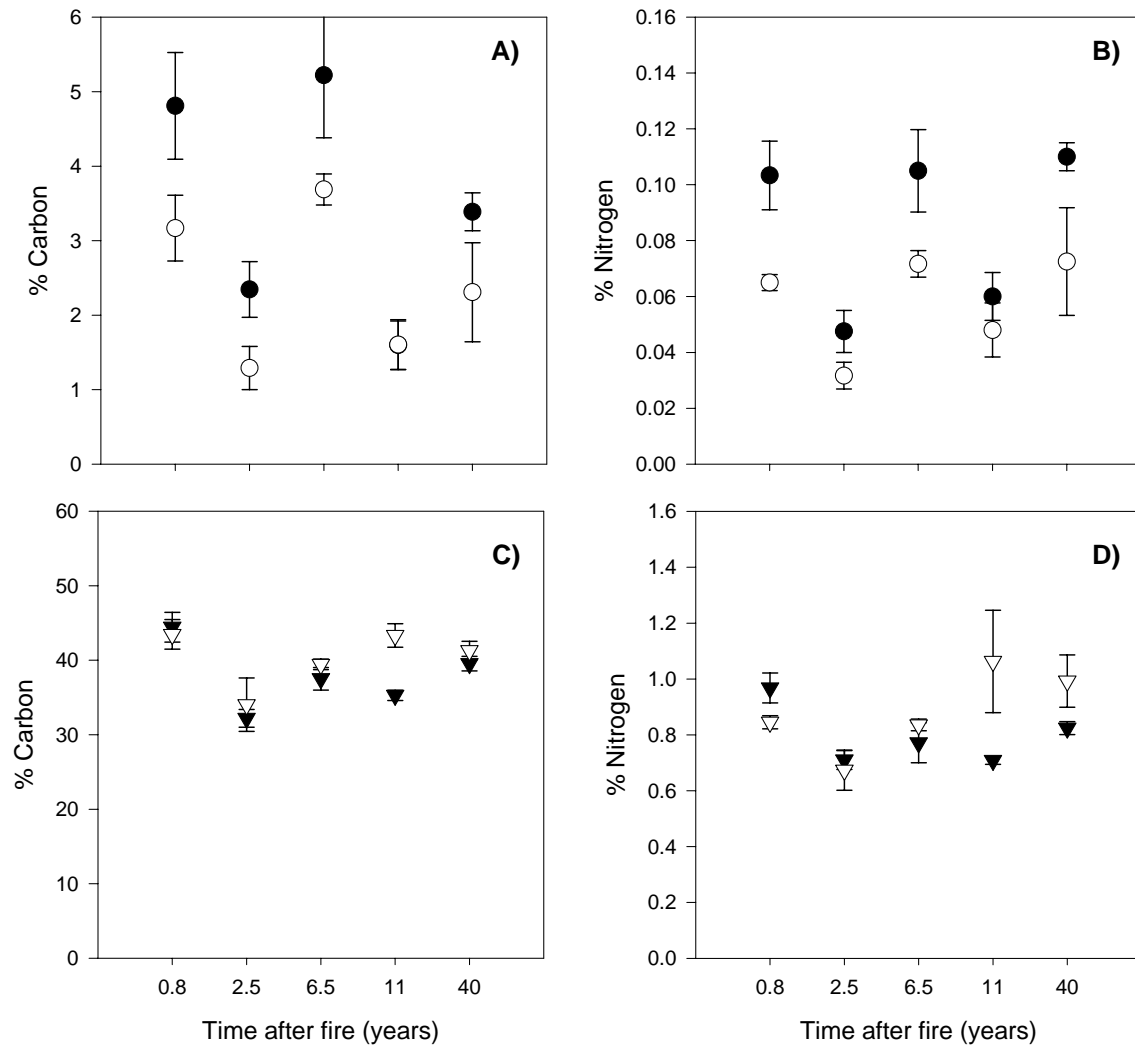


Figure 14: Carbon (A and C) and Nitrogen (B and D) content in dry mass basis in soil (●,○) and litter (▼,▽) samples for the long-term study. Closed signs correspond to samples collected in January and open signs correspond to samples collected in May; bars indicate standard error.

Community Level Physiological Profiling

The response of the microbial community to different substrates, on the parameters time to peak and peak amplitude presented variation among the four scenarios. Short term fire effects on microbial communities were evident on time to peak for soil and litter samples. PCAs factor scores were positively correlated with time to peak, indicating an increase in the time for the maximum substrate utilization immediately after fire (Figure 15-A and C) in the soil and litter samples. This effect was of relatively short duration on soil and litter microbial communities, and it disappeared by day 47 when time to peak decreased to pre-fire values. There were no particular effects over the 11 or 40 year old transects; the four burned transects were affected by the same magnitude, suggesting that the time elapsed previous to the last prescribed fire was not a determining factor of microbial community function.

No differences were observed in the long-term study for the soil and litter samples. The soil samples showed an increase in the variability on time to peak from January to May whereas the litter samples showed a clear increase on time to peak between January and May (Figure 16-A and C). Time to peak was shorter for January than May samples.

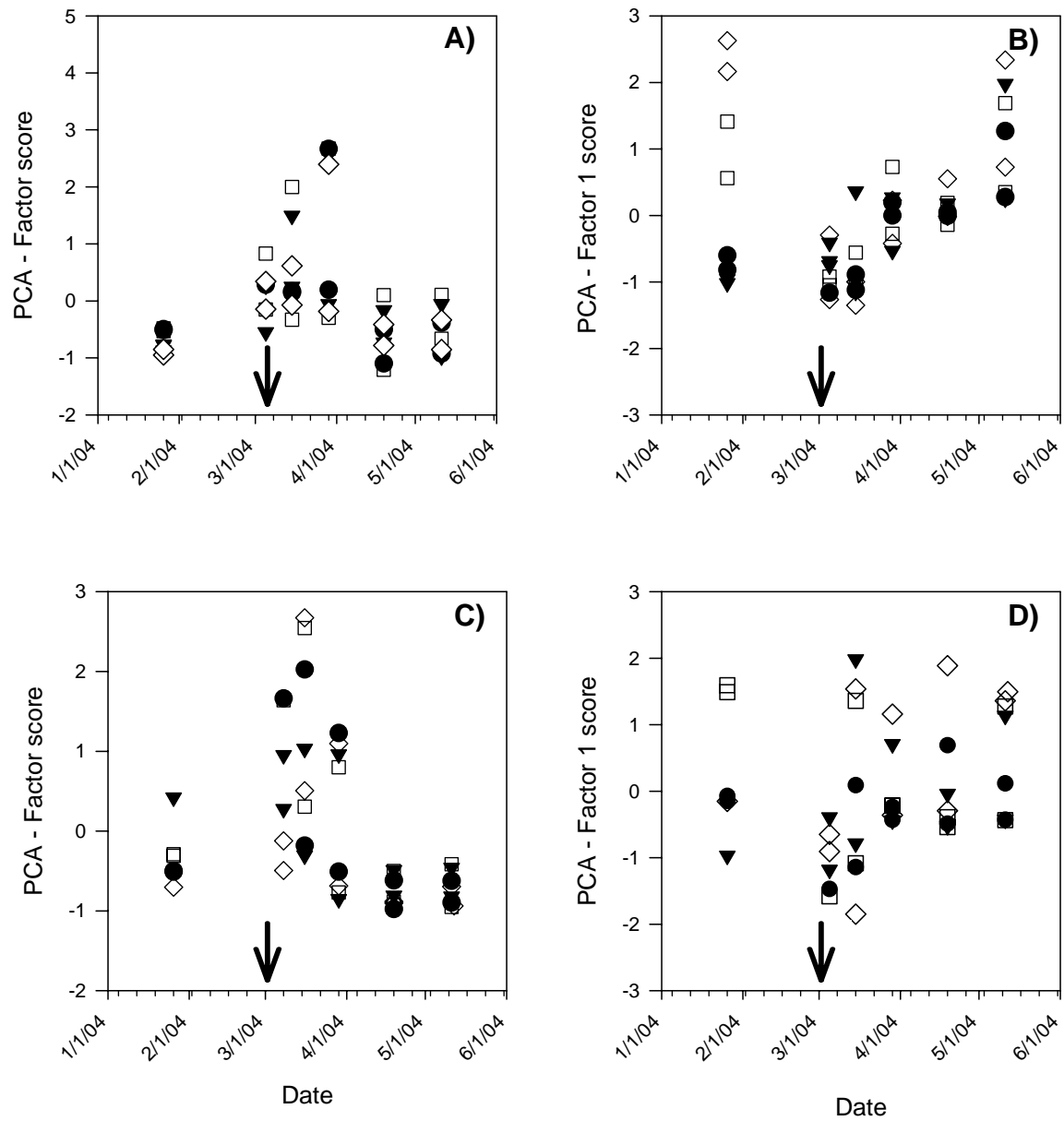


Figure 15: PCA factor scores for time to peak in soil (A) and litter (C) samples and peak amplitude in soil (B) and litter (D) from day 2 to day 70 after fire (short term) for 11 year (R20 ●, R21 ▼) and 40 year old transects (R25 □ and R26 ◇).

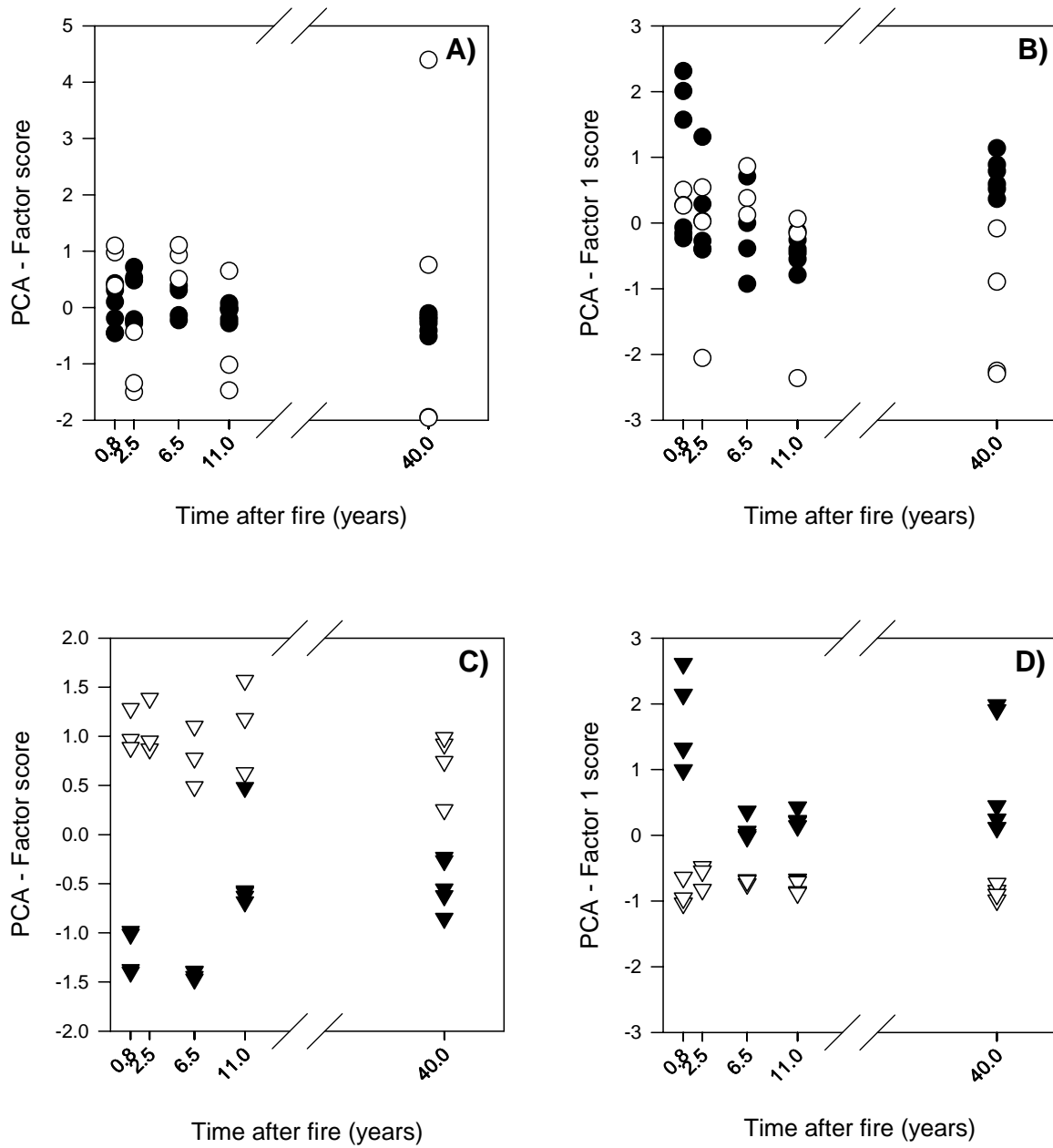


Figure 16: PCA factor scores for time to peak in soil (A) and litter (C) samples and peak amplitude for soil (B) and litter (D) from 0.8 to 40 years after fire (long term) during January (●-▼) and May (○-▽).

Peak amplitude showed different behavior in 11 and 40 year old transects. Before the fire occurred, the 40 year post-fire soil samples showed a higher peak amplitude in comparison with the 11 year post-fire soil samples (Figure 16-A). These differences were not as clear in the litter samples where the pre-fire samples did not differ as much in peak amplitude (Figure 16-C). However, after fire, the decrease in peak amplitude was more pronounced in the 11 year old than the 40 year old transect showing similar values immediately after fire. Peak amplitude progressively incremented after, and post-fire effects were overcome by day 69 (Figure 16-A). Litter samples showed a decrease in peak amplitude but the recovery in this parameter was more rapid than for the soil samples. Comparable post-fire values in peak amplitude to the pre-fire values were detected by day 10; these values presented high variability with no consistent differences between 11 and 40 year litter (Figure 16-C). For the long term study (Figure 16-B) there were no trends evident in the soil samples, only the 40 year old transect seemed to present differences between the January and May sampling, where the substrate utilization was higher in January than in March. Contrary to the soil, the litter samples showed a pronounced difference in the long term on peak amplitude. January peak amplitude was higher for all five transects (Figure 16-D), in agreement with previous evidence of seasonal effects over time to peak.

The individual performance of each substrate for both parameters, time to peak and peak amplitude, were compared for all four scenarios. The majority of the variability in time to peak was captured in one dimension whereas in the case of peak amplitude two axes were needed to capture all the variability of the data.

Table 4: PCA correlation coefficients of time to peak for soil and litter samples in the short-and long-term experiments. Percent values indicate the % of the variance captured in that particular Axis.

Substrate	Long Term		Short Term	
	Soil	Litter	Soil	Litter
	Axis 1 50 %	Axis 1 91 %	Axis 1 86 %	Axis 1 88 %
Citric acid	0.86	0.95	0.97	0.95
Mannose	0.95	0.98	0.97	0.96
Glucose	0.75	0.99	0.98	0.96
Xylose	-0.20	0.96	0.94	0.98
Inositol	0.95	0.92	0.95	0.96
Cas aa	0.00	0.94	0.97	0.92
Xylan	-0.63	0.92	0.82	0.84
0.8LE	0.75	0.97	0.96	0.93
11LE	0.95	0.92	0.96	0.95
40LE	0.05	0.97	0.97	0.95

Table 5: PCA correlation coefficients of peak amplitude for soil and litter samples in the short-and long-term experiments. Percent values indicate the % of the variance captured in that particular Axis.

Substrate	Long Term				Short Term			
	Soil		Litter		Soil		Litter	
	Axis 1 42 %	Axis 2 30 %	Axis 1 61 %	Axis 2 13 %	Axis 1 59 %	Axis 2 14 %	Axis 1 59 %	Axis 2 17 %
Citric acid	0.06	0.92	0.86	-0.33	0.78	-0.39	0.95	-0.06
Mannose	0.62	0.72	0.86	0.05	0.92	-0.25	0.92	-0.06
Glucose	0.79	0.38	0.86	-0.28	0.88	-0.21	0.81	0.26
Xylose	0.76	0.36	0.93	0.21	0.89	-0.11	0.88	-0.17
Inositol	-0.19	0.76	0.90	0.31	0.91	0.17	0.78	-0.41
Cas aa	0.66	-0.54	0.01	0.74	0.43	0.72	0.32	0.69
Xylan	0.68	-0.37	0.59	-0.44	0.45	-0.44	0.66	0.34
0.8LE	-0.63	0.61	0.88	0.33	0.91	0.22	0.63	-0.56
11LE	0.71	-0.01	0.49	-0.30	0.46	0.54	0.53	0.73
40LE	0.86	0.09	0.96	0.12	0.77	0.13	0.93	-0.12

All substrates except Xylose, Cas aminoacids and the 40LE, showed high correlation values with Axis 1, meaning that all of them showed a similar pattern. Xylan presented a high negative correlation with Axis 1, being the substrate upon which the microbial community showed a different pattern in time to peak (Table 4). Time to peak for most of the substrates (i.e glucose) showed smaller values and less variability within and among January than May samples. Meaning that the substrate utilization by the microbial community was more rapid and less variable for the same transect and did not show differences among the five different times to fire. Samples collected in May did not show a trend with time from fire; however, the microbial communities from all transects more slowly utilized the majority of the substrates (Figure 1- Appendix 3). Xylan utilization showed a different trend. January utilization was faster only for the 0.8 and 6.5 year transects, whereas the 2.5, 11, and 40 year old transects showed a faster Xylan utilization during the May sampling season.

Peak amplitude correlation coefficients (i.e., peak amplitude) did not show relevant differences among different scenarios for most of the substrates. However the 0.8LE, presented a highly negative correlation coefficient for the long term soil chronosequence. Therefore, the utilization trend among times after fire differed from that of most of the substrates (Table 5). January soil samples showed higher peak amplitude for most of the substrates (i.e. glucose) for the 0.8, 6.5 and 40 year old transects. Samples corresponding to 2.5 and 11 year transects did not show differences on peak amplitude between seasons. Peak amplitude of the 0.8LE was higher for those samples collected in May than for those collected in January (Figure 2 – Appendix 3)

The factor score value of each sample for time to peak and peak amplitude was plotted against the number of cells, to quantify density effects. The number of culturable cells was correlated negatively to the time to the maximum response in the short term study. However, this relationship was not found in the long term study for the soil or the litter. In the case of the peak amplitude there was no difference among scenarios, however this parameter was governed by the number of culturable cells present in the samples.

Paired T-tests were performed to analyze differences between time to peak in soil and litter samples. There were significant differences between the mannose and glucose for those samples collected in January. Litter samples showed consistently faster substrate utilization for all transects (Figure 3 – Appendix 3). The pattern was inversed for those samples collected in May. In this case there was a difference in substrate utilization for xylan, manose, xylose, casa aminoacids and the 40LE, where the soil microbial community displayed a faster utilization of the substrates (Figure 4 – Appendix 3). After fire samples were analyzed, the litter more slowly metabolized all the evaluated substrates (Figure 5 – Appendix 3)

CHAPTER FOUR: DISCUSSION

The plant community of the scrub ecosystem is dependent on fire to maintain ecosystem function, and it has been demonstrated to be resilient to fire (Schamlazer and Hinkle 1987 and 1996; Schmalzer 2003). However, the fire effects over the soil microbial component of the ecosystem seemed to be minor and of short duration.

The prescribed fire, represented by average high temperatures (DeBano et al. 1998), was spatially heterogeneous. This was accentuated in the 11-year old transects; the burned portions of the 40-year old transects were more homogeneous, which may have been caused by a more even distribution of the fuel (personal observation). Fire caused a decrease on soil carbon and nitrogen content for the 40-year old transects, which could be associated to oxidation and volatilization of these two nutrients due to the temperatures experienced during the prescribed fire (Raison et al. 1985). These nutrients reached pre-fire values rapidly. Even though, leaching has been considered a minor pathway of nutrient transport and loss after fire in other systems (Mackensen et al. 1996, Caldwell et al. 2000, Tremmel et al. 2004), the scrub ecosystem could represent a different response in this aspect. Nutrient inputs from the litter layer to the soil could be produced by the Florida summer showers, which constitute torrential precipitations, like that one occurred on March 16th when the precipitation was 15.74 mm and could certainly produce leaching of the soluble fractions of C and N. The retention of nutrients in this type of soil would be accomplished by the microbial community, generating a positive feedback (Singh et al. 1991, Jensen et al. 1991) preventing a net loss of nutrients from the ecosystem. Further microbial cells death and mineralization, would release these nutrients, and transfer to plant roots would facilitate plant re-growth. No trend in nutrient accumulation over the

long-term was observed, possibly explained by inherent site differences among the transects that negated the fire effect. The C:N ratio analysis seemed to indicate that there was not differential carbon or nitrogen accumulation after fire in the short-term of these two nutrients, they were incorporated to the litter and the soil in the same proportion. However, a lower C:N ratio was found in the soil when analyzed in the long-term. Goodale and Aber (2001) proposed the occurrence of decreasing soil C:N ratios through succession in eastern USA explained by nitrogen deposition, which could be possible in this coastal ecosystem. In addition to this, organic matter decomposition mediated by microorganisms has been largely demonstrated to affect C:N ratio.

Bacterial abundance was reduced by fire. But the effect of the fire seemed not to be a direct effect due to increases in the soil temperature as proposed by DeBano et al. (1998), if not by indirect effects or combined effects of fire and environmental factors. As previously suggested (Henrot and Robertson 1994, Díaz-Ravina et al. 1996, Dumontet et al. 1996, Ross et al. 1997) the accelerated desiccation of the upper layer and in this case intensified by the lack of precipitation could be the limiting factor for microbial activity. Soil and litter presented differences in percent water content and also in the range of variation. Therefore, the soil and the litter would represent two different environments in terms of water retention, the soil being more stable conditions, and the litter more variable. In the long term, the largest differences were due to the amount of precipitation, but also there was a considerable local variation. The 40-year old transects showed the largest water content loss, it possessed the highest water content in January and one of the lowest in May, this may have been caused by the increased atmospheric demand and

therefore a higher evapotranspiration exerted by a substantially larger saw palmetto canopy.

The number of culturable cells dramatically decreased by day 13 after fire and even though there was no decrease in water content at that particular time, the effect may be due to the prolonged occurrence of dry conditions. The number of culturable cells in the soil did not respond positively to the torrential precipitation on day 27, the cell counts were still lower by two orders of magnitude compared with the pre-fire samples. However, the microbial community seemed to recover rapidly from indirect fire effects after 49 days when the number of culturable cells reached the same order of magnitude of the pre-fire samples.

Microbial activity certainly was affected by the environmental conditions previously stated. Time to peak was a reliable parameter to detect disturbance and environmental stress effects over the microbial community as well as differential patterns in substrate utilization. Inoculum density had an impact on time to peak; the increment in number of microorganism was related to a decrease in the lag time. However, these relationships are only valid when analyzing differences within samples collected from one particular substrate, litter or soil. Litter samples presented higher culturable cell counts than the soil; however, the lag time for the litter samples was longer than for the soil samples, upon certain substrates. In the long-term study, January litter and soil samples presented differences the number of culturable cell counts by one order of magnitude whereas the differences for May were of two orders of magnitude. January soil and litter samples lag time did not differ for the majority of the substrates; however glucose and mannose were more readily utilized by the litter microbial community. The

situation was reversed in May where certain substrates (xylan, xylose, cas aminoacids and the 40LE) included mannose were more readily utilized by the soil microbial community than the litter microbial community. This demonstrated that this study was not only able to detect inoculum density effects but also differences in the physiological status of the microbial community as determining factors of the substrate utilization lag time. Hypothetical structural differences on the soil and litter microbial community would produce in differences in C sources utilization. However, this does not seem to be the case. The effect is not a total shift in the utilization of C sources if not a change on the magnitude of utilization. In addition if any structural change occurred, presumably the fungal component would increase in the litter microbial community compared to the soil. The type of substrate and the increased resistance of fungi to water stress compared to bacteria, would result in a shorter lag time for the litter than the soil samples under drought conditions. Nonetheless, it was the opposite. There are two potential explanations: differences in lag time would rely on differences on metabolic status of the microorganisms subject of drying-rewetting cycles in a very variable environment in the litter samples compared to the more stable environment in the soil. This argument though does not seem to be valid since the percent colony formation indicates that those microorganisms present in the litter samples in May, developed colonies more rapidly than in January. This would suggest a shift in microbial community structure towards r-type of organisms under stressing conditions (DeLeij FAAM et al. 1993, Garland et al. 2001) which would exert faster substrate utilization, particularly upon simple substrates. Mannose, xylose and cas aminoacids are simple substrates but the lag time for these substrates was longer in May than in January, invalidating the previous explanation. The

second explanation seems unlikely but still possible. Cyanobacteria are likely to be present in the soil surface in the scrub ecosystem (Norton and Davis 1975, Hawkes and Fletcher 2002) therefore we could expect to find them in the litter samples were they would be exposed to solar radiation. These would be capable to photosynthesize, even during the incubation of the plates since the wavelength of the light emitted by the fluorometer lamp (485 nm) would be enough to produce the excitation of the chlorophyll a, therefore this would reintroduce oxygen in the silicone matrix decreasing the fluorescent signal.

Xylan utilization by the soil microbial community presented an interesting pattern in the long-term. Its utilization occurred more rapidly in May than in January, contrary to the general response upon the rest of the substrates. This could be related to a higher content of xylan in the soil due to the oaks leaf abscission occurring in mid March. This would yield a relative increase of those microorganisms capable to utilize the xylan as a carbon source. Xylan is a complex polymer therefore the initial polymer breakdown would constitute a regulatory step for further degradation and utilization by non-xylan degrading microorganisms. This was evidenced on the 11 and 40 year old transects where the higher inputs of xylan from leaf fall would be expected.

In accordance with these findings, Zak et al. (1994) explained that the relatively large proportion of labile C in arid ecosystems would indicate that other factors such as soil water potential, are likely limit its in situ metabolism. Since the proportion of labile C is relatively large compared to the total soil pool (not quantified in this study), a change in climate, which would impact microbial activity, could potentially influence the capacity of this ecosystem to store C, and therefore produce changes in the C:N ratio

observable in the long term. In the scrub ecosystem, there is a significant seasonal effect on the soil microbial community, not only explained by changes in soil water potential but also associated with the relationships among precipitation (Wardle 1992), plant canopy evapotranspiration (Zak et al. 1994), soil temperature (not analyzed in the present study), and substrate input. This apparently represents a biological constraint over the function developed by the microbial community, quantitative and qualitative speaking.

Biological constraints could also be represented by the overlaying plant community. Oak-saw palmetto scrub is basically composed of clonal plants, which resprout in response to fire. Eriksson (2000) has coined the term “remnant populations” to refer to those populations that persist in the environment without completion of the whole life cycle, implementing different strategies such as having long vegetative phases. These species have the capacity to recover and return to the conditions prior to the disturbance. The clonal nature and the proven resilience (Schmalzer and Hinkle 1987) of the scrub plant community make a good case for the individual species classification as “remnant populations”. Remnant populations increase the stability of the ecosystem that the species is part of (Eriksson 2000) through interactions with microorganisms, regulating/ buffering nutrient availability. Also, in the particular case of the scrub species, the reduced genetic variability in comparison with seeding plants could add to the specificity of the plant-microorganism relationships. Plant species would provide with a more stable environment, where the success of the microbial community resides in the ability to overcome hydric stress. The microbial community apparently develops crucial roles in nutrient “trapping” in a particularly nutrient starved ecosystem adding to the stability of the ecosystem. The strong stabilizing effect of the vegetation and the frequent

disturbance coupled with extremely low water availability, determine a similar niche breadth observed on the lack of change on soil and litter physiological profiling. The community-level physiological profiling did not show shift in substrate utilization pattern, it only showed quantitative changes related to inoculum density and possibly physiological status. Therefore soil microbial community dynamics in the oak-saw palmetto scrub ecosystem was determined by the soil water content, and the extreme variability on this parameter was a stronger disturbance than the fire itself.

Methodological considerations

The pH of the samples during the incubation was regulated with a buffer at a higher pH than occurring in the soil or litter sample, probably affecting the microbial substrate utilization. Schmalzer and Hinkle (1991) detected an increase in soil pH after fire, and post-fire microbial communities could become acclimated, therefore potentially causing a differential effect on the samples over time.

CHAPTER FIVE: CONCLUSION

This study suggests that fire effects on the soil microbial community of the scrub ecosystem are ephemeral and dictated by stringent environmental conditions. These two factors affected quantitatively the microbial community and its physiological response. Structure and function of the microbial community seemed to be governed by water availability and the frequency of the water input which ultimately determined differential nutrient utilization efficiencies in the soil and litter layer. It seems appropriate to refer to the plant community, particularly the oak species and saw palmetto as remnant populations which would confer stability to this ecosystem. The biological stabilizing effect of the vegetation and the frequent disturbance existing in extreme water conditions over short periods of time, would determine a similar niche breadth over long periods of time observed on the lack of qualitative changes on soil and litter physiological profiling.

Even though our results on fire effects are conclusive, variability among the sites brought difficulties to the analysis of nutrient dynamics. Therefore it would be appropriate to follow up site by site, to better characterize nutrient dynamic and microbial substrate utilization efficiency in the long term.

The BD Oxygen-biosensor system demonstrated to be a reliable methodology in constructing the community physiological profiling parameter detecting not only disturbance effects, but also environmental stress effects on the soil and litter microbial community. Future research should explore the use of this tool to discriminate the metabolic activity exerted by various fractions of the microbial community as fungi and cyanobacteria. Even though there is no evidence to lead to shifts in compositional

changes of the microbial community this is one of the aspects that further research should address.

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APPENDIX A
CLIMATE DATA

TABLE A-1: Climatic data for the sampling season and month averages for the decade. Weather data were recorded in the Shuttle

Landing Facility, Cape Canaveral.

DATA	JAN.	Month av.	FEB.	Month av.	MARCH	Month av.	APRIL	Month av.	MAY	Month av.
EXTREME MAX TEMP	26 (C)	29 (C)	28 (C)	31 (C)	29 (C)	32 (C)	29 (C)	33 (C)	35 (C)	34 (C)
MEAN MAX TEMP	20 (C)	21 (C)	21 (C)	22 (C)	24 (C)	24 (C)	25 (C)	26 (C)	29 (C)	29 (C)
MEAN TEMP	14 (C)	16 (C)	16 (C)	17 (C)	19 (C)	19 (C)	20 (C)	21 (C)	24 (C)	24 (C)
MEAN MIN TEMP	9 (C)	10 (C)	11 (C)	12 (C)	13 (C)	14 (C)	14 (C)	16 (C)	19 (C)	19 (C)
EXTREME MIN TEMP	2 (C)	-7 (C)	3 (C)	-1 (C)	7 (C)	-2 (C)	7 (C)	4 (C)	12 (C)	8 (C)
HEATING DEGREES	242	197 (Days)	153	124 (Days)	46	69 (Days)		14 (Days)	0	1 (Days)
COOLING DEGREES	14	44 (Days)	26	63 (Days)	60	109 (Days)		177 (Days)	312	322 (Days)
EXTREME MAX RH	100 (%)		100 (%)		100 (%)		100 (%)		100 (%)	
MEAN MAX RH	99 (%)		98 (%)		95 (%)		95 (%)		94 (%)	
MEAN RH	81 (%)	80 (%)	87 (%)	76 (%)	76 (%)	75 (%)	75 (%)	72 (%)	74 (%)	75 (%)
MEAN MIN RH	55 (%)		68 (%)		54 (%)		55 (%)		55 (%)	
EXTREME MIN RH	26 (%)		41 (%)		25 (%)		32 (%)		42 (%)	
MEAN WIND	6 (KTS)	7 (KTS)	6 (KTS)	7 (KTS)	7 (KTS)	8 (KTS)	7 (KTS)	7 (KTS)	6 (KTS)	6 (KTS)
PEAK WIND	34 (KTS)	46 (KTS)	38 (KTS)	60 (KTS)	33 (KTS)	48 (KTS)	36 (KTS)	53 (KTS)	35 (KTS)	46 (KTS)
MEAN DIRECTION	249 (Deg)	330 (Deg)	213 (Deg)	000 (Deg)	157 (Deg)	150 (Deg)	178 (Deg)	150 (Deg)	145 (Deg)	120 (Deg)
24 HR MAX PRECIP	24.13 (mm)	115.57 (mm)	42.67 (mm)	56.39 (mm)	15.75 (mm)	95.00 (mm)	13.46 (mm)	130.30 (mm)	42.42 (mm)	61.47 (mm)
PRECIP TOTAL	58.42 (mm)	66.80 (mm)	88.14 (mm)	66.04 (mm)	16.25 (mm)	88.90 (mm)	30.73 (mm)	70.10 (mm)	48.26 (mm)	74.42 (mm)
EXTREME MIN PRECIP		1.52 (mm)		2.29 (mm)	0.00 (mm)	19.56 (mm)	(mm)	1.02 (mm)	(mm)	21.59 (mm)
PRECIP DAYS (>0.01 in)	6	7	6	7	2	7	6		3	8
TSTM DAYS	3	1	1	2	1	3	4		3	7
FOG DAYS	8	13	9	10	9	9	7		15	7

APPENDIX B

C:N REGRESSION ANALYSIS

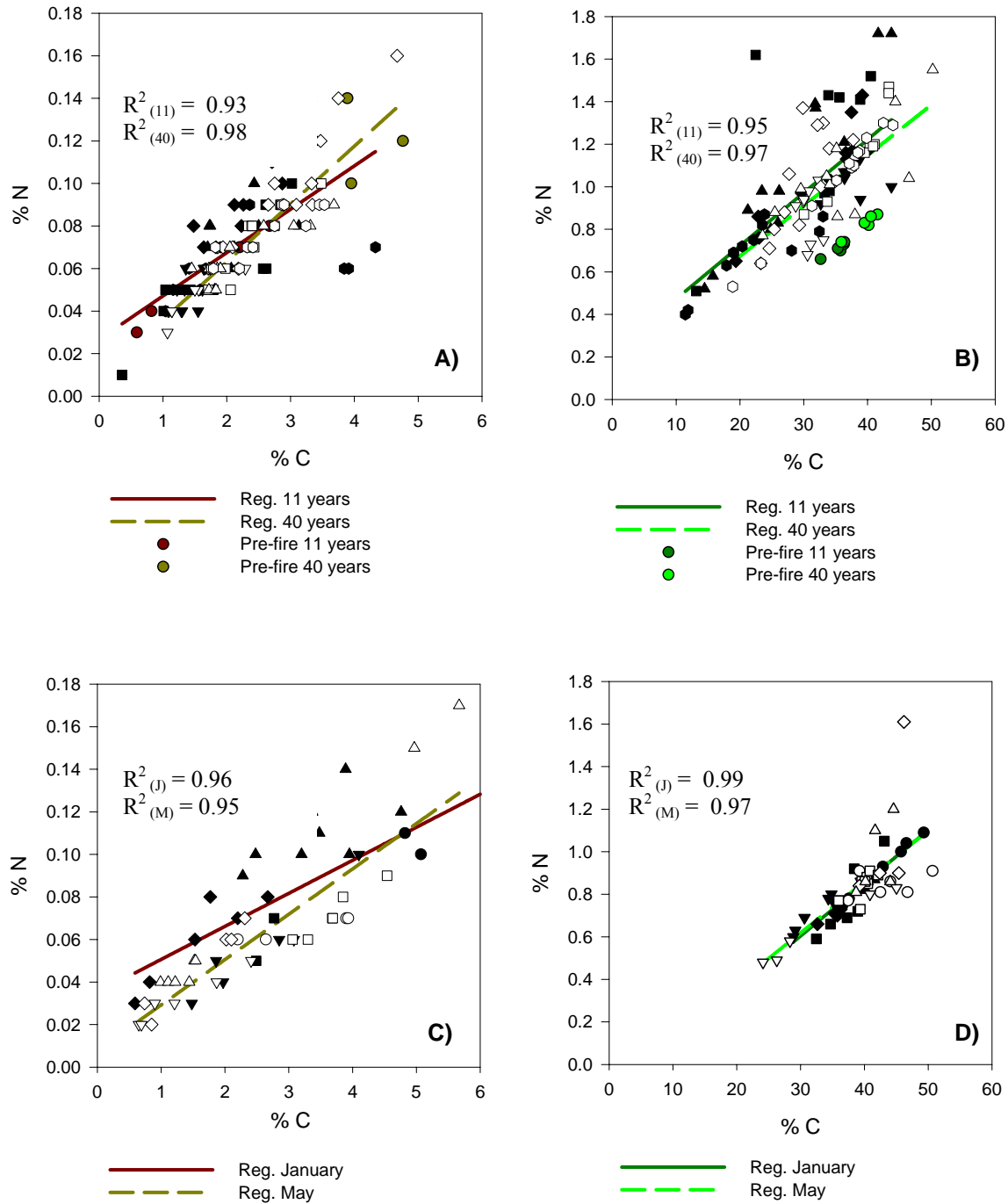


Figure 1-B: Regression analysis for C:N ratio for the 4 scenarios. Soil A), and B) litter short-term experiment. The colored symbols correspond to the pre-fire samples, black for the 11 and white for the 40 year old transects. Sampling dates represented as follows: ▼ day 2, ■ day 13, ◇ day 27, ▲ day 49 and ● day 70. Soil C), and litter D)

long-term experiment. Black symbols represent January sampling and white symbols represent May sampling. Different FSP are noted as follows: \blacklozenge 0.8, \blacktriangledown 2.5, \blacksquare 6.5, \diamond 11 and \blacktriangle 40 years FSP.

Table 1-B: Regression analysis parameters for the four studied scenarios.

Substrate	Study	Sample	Parameter	Value	Std. error	t	P
Soil	Short Term	11years	y_0	0.027	0.006	4.39	<0.0001
			a	0.020	0.003	7.18	<0.0001
		40 years	y_0	0.009	0.004	1.99	0.0507
			a	0.027	0.002	16.59	<0.0001
	Long Term	January	y_0	0.035	0.007	4.78	<0.0001
			a	0.016	0.002	8.40	<0.0001
		May	y_0	0.008	0.006	1.41	0.171
			a	0.021	0.002	10.45	<0.0001
Litter	Short Term	11years	y_0	0.223	0.111	2.01	0.0492
			a	0.025	0.004	7.15	<0.0001
		40 years	y_0	0.199	0.122	1.64	0.1077
			a	0.024	0.004	6.86	<0.0001
	Long Term	January	y_0	-0.143	0.084	-1.72	0.0982
			a	0.025	0.002	11.31	<0.0001
		May	y_0	-0.102	0.201	-0.51	0.6173
			a	0.024	0.005	4.82	<0.0001

APPENDIX C

SUBSTRATE UTILIZATION PATTERNS

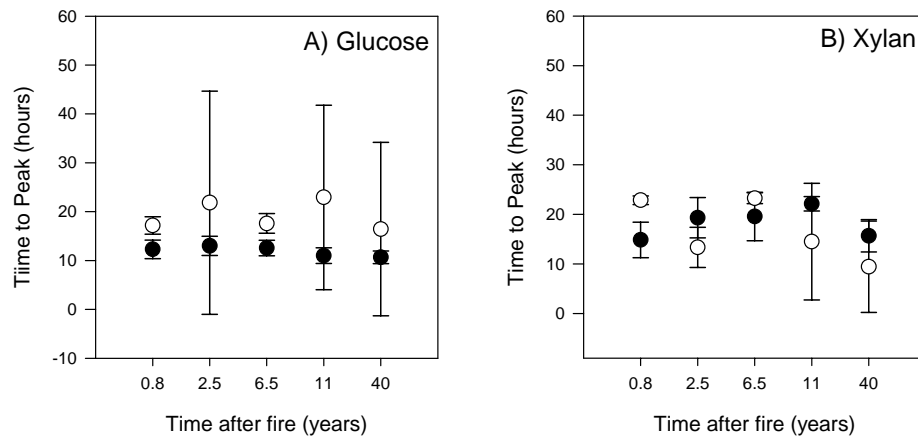


Figure 1-C : Soil microbial community time to peak for two substrates A) glucose showing the general trend, and B) xylan which deviated from the general trend, during January (●) and May (○) sampling seasons.

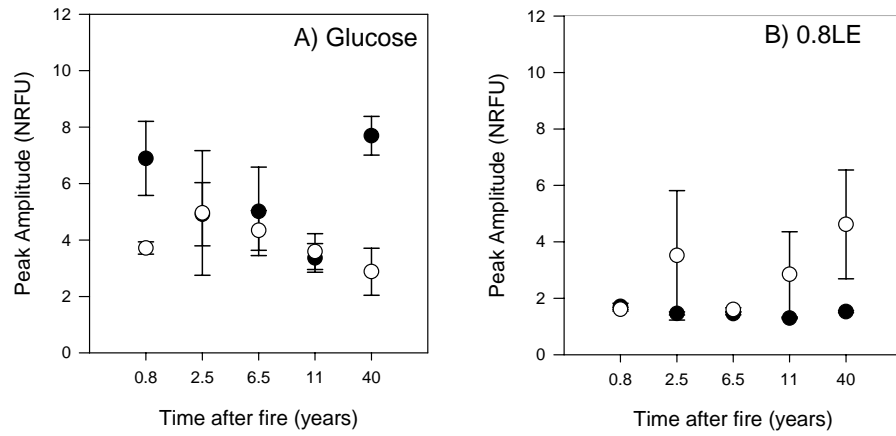


Figure 2-C: Soil microbial community peak amplitude for two substrates A) glucose showing the general trend, and B) 0.8LE which deviated from the general trend, during January (●) and May (○) sampling seasons.

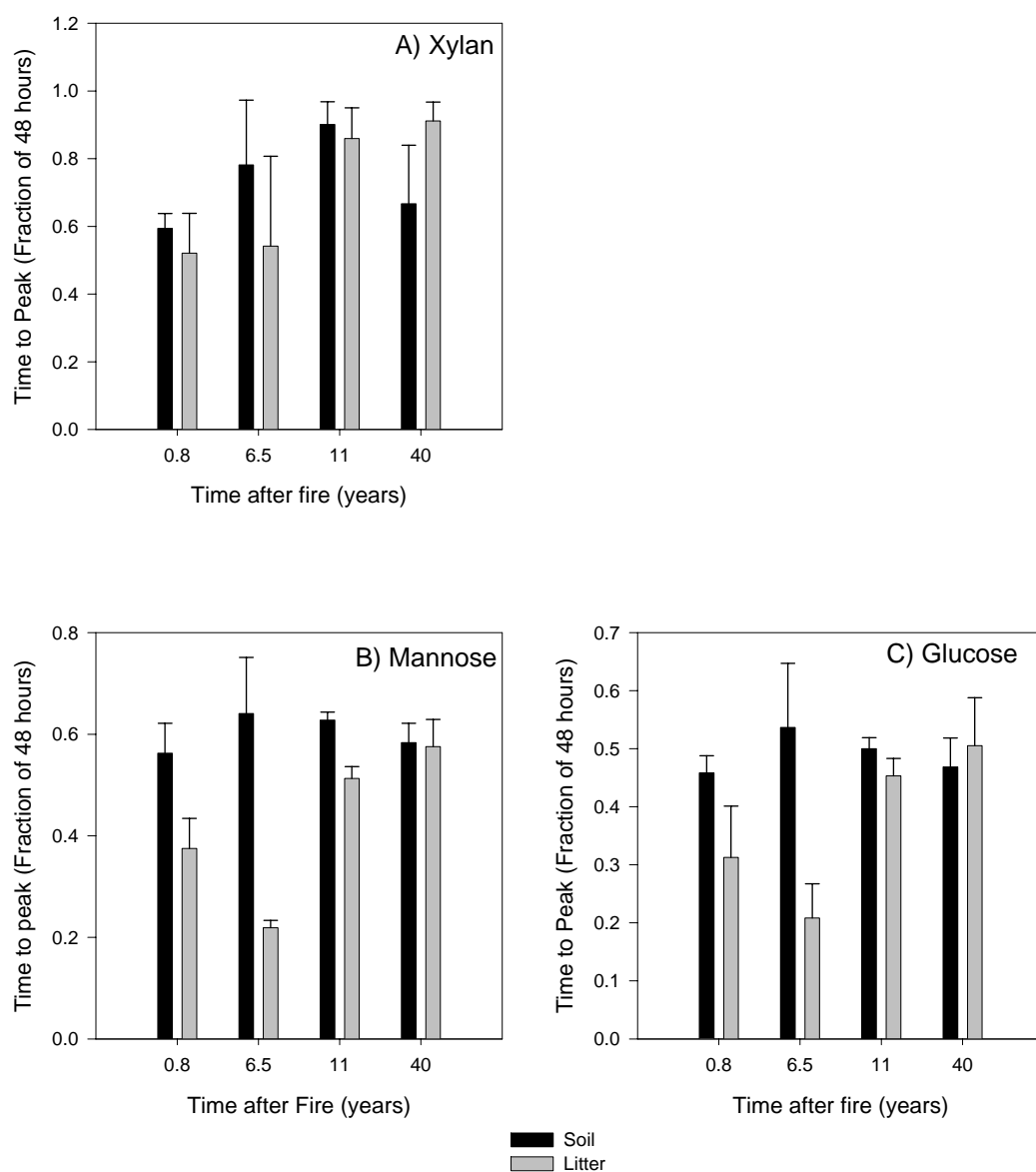


Figure 3-C: Time to peak for soil and litter samples for January sampling season. A) xylan: represents the no-difference scenario. B) Mannose, and C) glucose represent the substrates for which there were significant differences between soil and litter samples (Paired t-test). Bars indicate standard deviation.

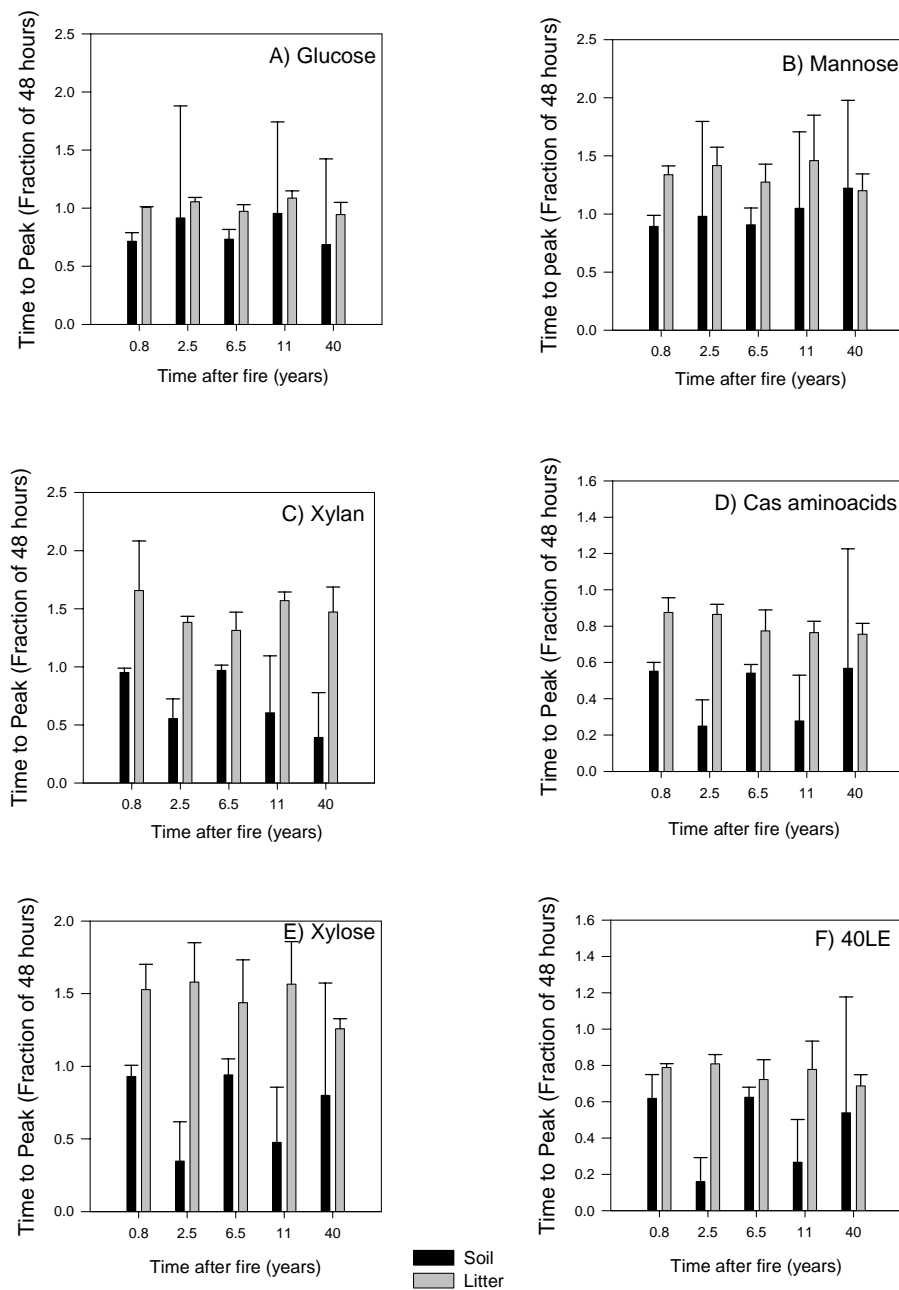


Figure 4-C: Time to peak for soil and litter samples for May sampling season. A) Glucose represents the no-difference scenario. B) Mannose, C) xylan, D) cas aminoacids, D) xylose, and E) 40LE represent those substrates for which there was significant differences between soil and litter samples (Paired t-test). Bars indicate standard deviation.

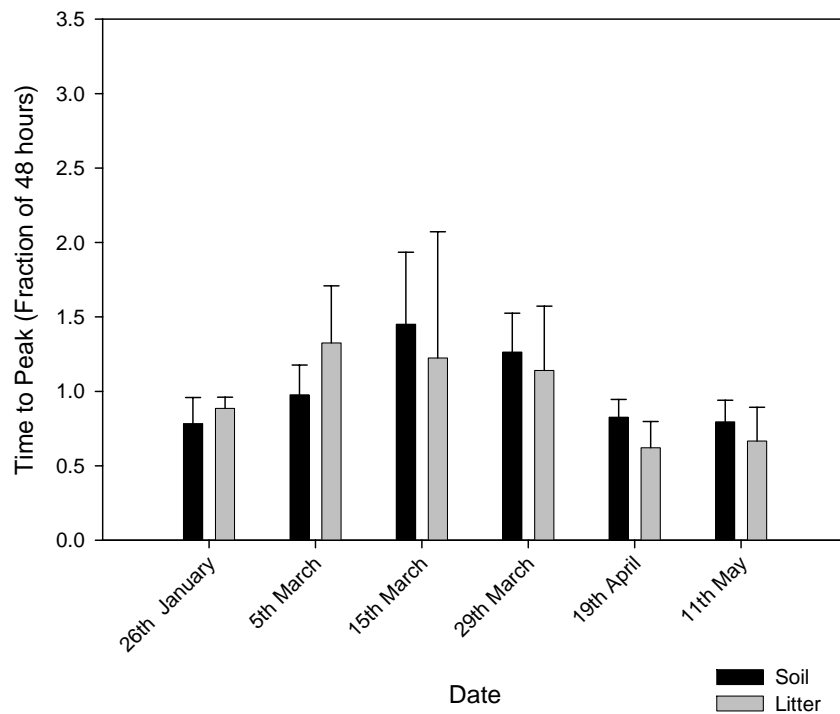


Figure 5-C: Time to peak for xylan in soil and litter samples for the short-term experiment. This is a sample graph representing all the substrates which showed significant differences between soil and litter samples (Paired t-test). Bars indicate standard deviation.